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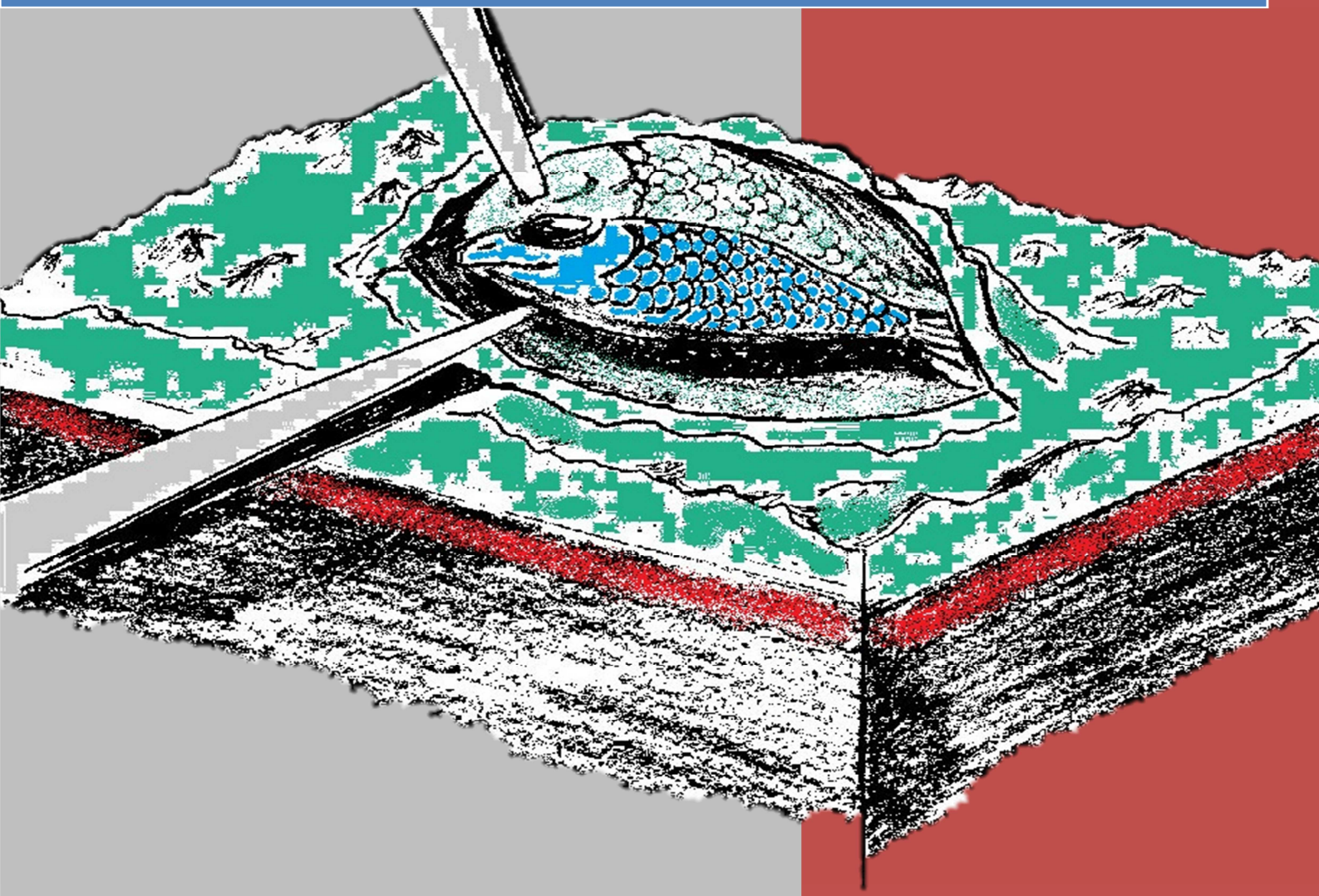
MICROBIAL MATS: THE IMPLICATION OF THESE MICROBIAL COMMUNITIES IN EARLY STAGES OF FOSSILIZATION



Miguel Iniesto Rodríguez

Tesis Doctoral

MICROBIAL MATS: THE IMPLICATION OF THESE MICROBIAL COMMUNITIES IN EARLY STAGES OF FOSSILIZATION

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L'influence des microorganismes sur la qualité de la fossilisation est essentielle. Doués d'un extraordinaire pouvoir de prolifération, ils colonisent presque instantanément les milieux lorsque les conditions leur deviennent favorables. Aussi offrent-ils des signaux paléoécologiques très sensibles. Pour cerner leur impact sur la sédimentation et sur la fossilisation, une étroite collaboration est nécessaire entre géologues et microbiologistes, afin de reconnaître dans les formations sédimentaires anciennes la nature des écosystèmes microbiens et celle des métabolismes mis en jeu » Impact des écosystèmes microbiens sur la sédimentation.

Jean Claude Gall et al.
Palaeoclimatol. Palaeoecol 111:17-28

La influencia de los microorganismos en la calidad de preservación es esencial. Dotados de un extraordinario poder de proliferación, colonizan de forma casi instantánea cualquier medio en el que las condiciones les sean favorables. Además ofrecen señales paleocológicas muy sensibles. Para determinar su impacto en la sedimentología y la paleontología, es necesaria una estrecha colaboración entre geólogos y microbiólogos, con el fin de reconocer la naturaleza de los ecosistemas microbianos y los metabolismos puestos en juego en las estructuras sedimentarias primitivas

Jean Claude Gall y colaboradores
Palaeoclimatol. Palaeoecol (1994) 111:17-28

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Aunque nunca es fácil plasmar en palabras lo que se siente o lo que se quiere decir, hay dos personas a las que les quiero agradecer toda la dedicación y tiempo que han pasado conmigo. **Anabel** y **MªCarmen**, mis directoras, que son las personas que han hecho posible que esto sea posible. Cuando llegué a su laboratorio de Ecología Microbiana a mediados de 2008 yo era un estudiante perdido sin un proyecto claro para realizar su PFC, y aquí me encuentro escribiendo los agradecimientos de mi tesis… Y todo gracia a ellas. Ellas son las que me han formado como científico, las que me han enseñado lo increíble que es esta profesión y las que me han dado las fuerzas y energías para realizar este trabajo. Todo doctor/doctorando sabe lo dura que puede llegar a ser esta etapa, pero con unas directoras como Anabel y MªCarmen al lado todo se hace más fácil. Han sabido guiarme cuando lo he necesitado y ayudado siempre que ha hecho falta, pero sin ser agobiantes o dominantes. Aunque nada de esto es nuevo para aquellos que han pasado esta etapa a mi lado, porque siempre que tengo la ocasión presumo de las directoras que tengo: MUCHAS GRACIAS JEFAS!

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A **Anabel** (de nuevo) por la ilustración de la portada.

RESUMEN

Durante décadas la paleontología ha tratado de establecer los mecanismos que pudieran explicar la génesis de los Konservat-Lagerstätten, yacimientos paleontológicos que se caracterizan por la excepcional preservación de sus restos. Estos fósiles mantienen en algunos casos tejidos lábiles difíciles de preservar como piel, sangre u órganos internos, así como un nivel de articulación muy superior al de los procedentes de yacimientos convencionales. Este excelente estado de conservación ha sido frecuentemente explicado por la comunidad científica en base a la presencia y actividad de tapetes microbianos, complejas comunidades multilaminadas de microorganismos, aunque su implicación no había sido demostrada experimentalmente de forma sistemática. En esta tesis se presentan una serie de experimentos multidisciplinares de larga duración (hasta 5.5 años) que tratan de demostrar y explicar la participación de los tapetes microbianos en las etapas tempranas de la formación de fósiles. Mediante el uso de métodos de análisis muy diversos, como microelectrodos, resonancia magnética, microscopía electrónica o espectroscopía Raman, se ha comprobado que la presencia de estas comunidades retrasa la descomposición de invertebrados como artrópodos y vertebrados como peces y anuros. Tras la colocación de los cadáveres en la superficie del tapete, éste comienza un proceso de reestructuración que desemboca en el recubrimiento del cuerpo y la formación de un sarcófago protector. En el interior de esta envoltura, el cadáver se encuentra aislado y expuesto a condiciones físico-químicas que difieren del exterior debido a la actividad de las poblaciones microbianas. El sarcófago, que en su cara interna está formado por células de pequeño tamaño embebidas en una matriz de polisacáridos y proteínas, permite que se produzcan tres tipos de rutas para la fosilización: 1) la preservación orgánica de los tejidos propios del cadáver; 2) la mineralización y 3) la generación de impresiones y réplicas. La primera consiste en que los tejidos orgánicos, en vez de ser descompuestos rápidamente, perduran a lo largo del tiempo manteniendo gran parte de su estructura y composición. Aunque este mecanismo es extremadamente raro y controvertido, parece encontrarse en el origen de la preservación de ciertos fósiles excepcionales. En presencia de los tapetes, los cadáveres ven ralentizada de forma significativa su descomposición, por lo que la preservación de los tejidos es más probable. De hecho, se ha comprobado la existencia de órganos internos en moscas y peces tras 5 años y la presencia de músculo y tendones en ranas después de 3 años. En segundo lugar se encuentra la generación de minerales que sustituyen los tejidos originales. El microambiente del interior del sarcófago, predominantemente básico y óxico, así como las propiedades inherentes de los EPS que actúan como

captadores de iones, favorecen la formación de diferentes fases minerales. Esto explicaría la detección de un precipitado rico en sílice y magnesio, similar al talco, recubriendo la cara superior en peces de 5 años y sustituyendo estructuras óseas como las aletas o la mineralización en carbonato de calcio de una región del cerebro medio en ranas de 3 años. Por último, además de la preservación de los tejidos originales de los cadáveres, ya sea mediante la ralentización de la descomposición o la mineralización, los tapetes microbianos son también capaces de generar impresiones que copian con gran fidelidad la superficie de los restos. Así, se ha podido observar que en el interior del sarcófago se preservaban las impresiones en negativo de las protuberancias del tegumento de anuros, el dibujo de la superficie de las escamas de pez o las sétulas y/o escamas del ala en el caso de las moscas. En el estudio del registro fósil estas impresiones son especialmente útiles para la recreación de tejidos externos como la piel, o para deducir la existencia de estructuras blandas como la bolsa gular de algunos dinosaurios. Los experimentos presentados en esta tesis, por lo tanto, aportan una base experimental sólida que confirma, completa y articula el papel de los tapetes microbianos en la formación de fósiles excepcionales y en consecuencia, en la génesis de algunos Konservat-Lagerstätten.

Palabras clave: **fosilización; mineralización; preservación excepcional; preservación orgánica; tafonomía; tapetes microbianos.**

ABSTRACT

Palaeontology has focused in understand the genesis of Konservat Lagerstätten over decades. These sites are characterized by the exceptional preservation of fossils, which maintain in occasions labile tissues, such as skin, blood or inner organs, as well as an articulation higher that fossils belonging to regular sites. Scientific community has frequently explained exceptional preservation by, among other factors, the presence of microbial mats, complex multi-layered microscopic communities. However, this relation lacked of a systematic actual experimental support. The present work exposes a battery of multidisciplinary and long-standing (up to 5.5 years) experiments showing and explaining the effect of mats in early fossilization. The impact of these communities in arthropods, fish and anuran decay has been highlighted with very diverse analytic methods, such as microsensors, magnetic resonance, electronic microscopy or Raman spectroscopy. The placement of carcasses over the mat surface leads to the reorganization of the upper layers and the coverage of bodies. Inside the sarcophagus animals are isolated and microenvironmental conditions are determined by the microbial population's activity. This sarcophagus, whose inner face is manly formed by small cells embedded in a polysaccharides and proteins matrix, favours the preservation of remains by three different pathways: 1) organic preservation of tissues; 2) authigenic mineralization and; 3) impression and replicates formation. The first one required a delay in decay of tissues, which maintained both organization and composition over large periods. Although this process is extremely rare and controversial, it is a key mechanism for exceptional preservation. Decay is significantly delayed inside mats, allowing the preservation of tissues. In fact, the presence of inner organs in flies and fish after 5 years and muscle, tendons and bone marrow in 3-years frogs has been detected with SEM. The second process is at the base of the substitution of original tissues by minerals. The occurrence of minerals can be explained by the environment inside the sarcophagus, mainly basic and oxic, as well as the inherent properties of the EPS matrix trapping ions and acting as nucleation site. In fact, the present thesis reports the existence of a talc-like Mg-rich silicate replacing bones as fins and covering the upper face of fish after 5 years or the mineralization in calcium carbonate of the midbrain in frogs after 3 years. Lastly, impressions and replicates are able to fossilize external morphologies of carcasses with remarkable fidelity. The inner face of the sarcophagus showed to generate reliable copies in negative of protuberances of the frog skin, the concentric shape of fish scales and setulae and/or wing scales of flies. The study of this type of impressions is useful to reconstruct external tissues, such as skin, and/or to know the existence of soft anatomical structures as the

throat pouch of several dinosaurs. The experiments exposed in the present thesis support with strength the impact of mats in early fossilization, and thus in the genesis of several Konservat Lagerstätten.

Keywords: **exceptional preservation; fossilization; microbial mats; mineralization, organic preservation; taphonomy.**

CONTENIDO

Página

LISTA DE ABREVIATURAS

Esta sección presenta el compendio de las abreviaturas que se emplean a lo largo de la presente tesis.

Abreviatura	**Término**
e.g.	Del latín *exempli gratia*, puede traducirse como “por ejemplo”
i.e.	Del latín *id est*, puede traducirse como “es decir”
et al.	Del latín *et alii*, puede traducirse como “y colaboradores”
EPS	Sustancias exopliméricas (del inglés *exopolymeric substances*)
UV	Ultravioleta
pH	Potencial de Hidrógeno
Ma	Millones de año
MISS	Estructuras sedimentarias inducidas por microorganismos (del inglés *microbially induced sedimentary structures*)
SEM	Microscopio electrónico de barrido (del inglés *scanning electron microscope)*
TEM	Microscopio electrónico de transmisión (del inglés *transmission electron microscope)*
EDXS	Energy-dispersive X-ray spectroscopy
DRX/XDR	Difracción de rayos X
rDNA	DNA ribosomal
DO	Oxígeno disuelto (del inglés *Dissolved oxygen*)
RMI	Imagen mediante resonancia magnética
Eh	Potencial REDOX
M.O.	Materia orgánica
RMN	Resonancia magnética nuclear (del ingés Nuclear magnetic resonance)

La presente tesis doctoral se presenta en forma de compendio de artículos. A continuación se detalla la información relativa a cada uno de los capítulos:

Capítulo 2: publicado

Iniesto M, López-Archilla AI, Fregenal-Martínez MA, Buscalioni ÁD, Guerrero MC. 2013. Involvement of microbial mats in delayed decay: an experimental essay on fish preservation. *Palaios*. 28(1):56–66. DOI: 10.2110/palo.2011.p11-099r

Capítulo 3: manuscrito en preparación

Capítulo 4: manuscrito en revisión en Scientific Reports (grupo Nature)

Iniesto M, Buscalioni AD, Guerrero MC, Benzerara K, Moreira D and López-Archilla AI. Exceptional preservation of vertebrates and invertebrates mediated by the formation of a sarcophagus and the body impression in microbial mats

Capítulo 5: publicado

Iniesto M, Laguna C, Florín M, Guerrero MC, Chicote Á, et al. 2015a. The impact of microbial mats and their microenvironmental conditions in early decay of fish. *Palaios.* 30:792-801. DOI: http://dx.doi.org/10.2110/palo.2014.086

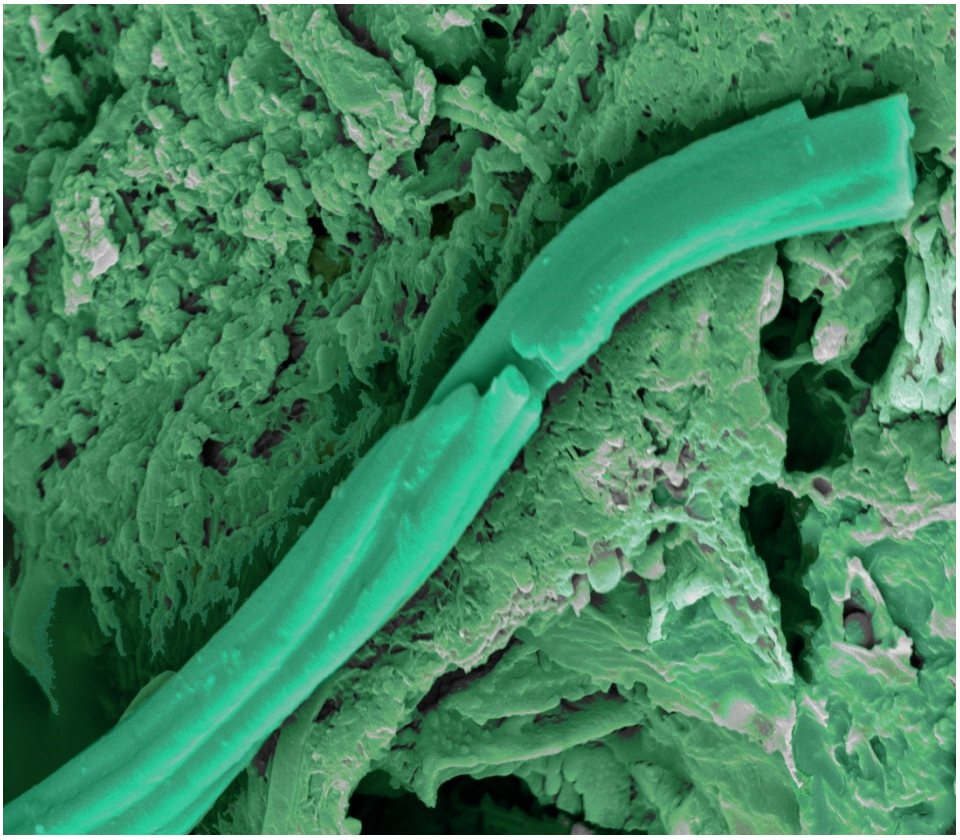
Capítulo 6: publicado

Iniesto M, Zeyen N, López-Archilla AI, Bernard S, Buscalioni ÁD, Guerrero MC and Benzerara K. 2015. Preservation in microbial mats: mineralization by a talc-like phase of a fish embedded in a microbial sarcophagus. *Front. Earth Sci.* 3:1-13. DOI: 10.3389/feart.2015.00051

El contenido de los capítulos 1 (Introducción) y 7 (Discusión) serán empleados para la elaboración de una futura publicación a modo de revisión

CAPÍTULO 1

INTRODUCCIÓN GENERAL



ANTECEDENTES

El estudio del registro fósil es una de las vías más fiables para comprender y conocer la evolución de la vida en la Tierra y los ecosistemas del pasado. En este contexto, ciertos yacimientos paleontológicos que han permitido la obtención de fósiles excepcionalmente preservados representan una de las fuentes más valiosas de información. Estos depósitos, denominados Konservat-Lagerstätten (Seilacher 1970), han supuesto una extraordinaria contribución para la documentación de los principales cambios evolutivos y biogeoquímicos a través de la historia geológica (Butterfield 2003), en gran parte gracias al sorprendente grado de conservación de los fósiles, llegando incluso a preservar tejidos blandos que normalmente se degradarían en las etapas tempranas que suceden tras la muerte del animal. La Tafonomía, como ciencia que trata de describir y explicar la fosilización, encuentra en los Konservat-Lagerstätten un gran desafío, especialmente porque su origen es variado estando con frecuencia asociados a sistemas lacustres o marinos someros y más raramente a permafrost, ámbar, arenas bituminosas, o turberas (Allison 1988). Adolf Seilacher, uno de los paleontólogos y tafónomos más reconocidos, dedicó su extensa carrera científica a intentar revelar los secretos que subyacían en la formación de estos yacimientos. Tras un exhaustivo análisis de los restos de muy diversos Konservat-Lagerstätten, Seilacher y colaboradores (1985) identificaron tres agentes primarios independientes, aunque no excluyentes, que explicarían su génesis, uno de los cuales sería la presencia de tapetes microbianos. Según los autores, estas comunidades promoverían la diagénesis temprana y facilitarían la preservación excepcional. En ese mismo año y en paralelo, Jean Claude Gall, tras el estudio de diversos fósiles y de los sedimentos que los contenían, coincidía en proponer que unos "velos microbianos" estarían en el origen de algunos fósiles excepcionales. A pesar del importante papel atribuido a los tapetes, el cómo éstos llevarían a cabo o influirían sobre los procesos de fosilización carecía en gran medida de base experimental, llegando incluso a ser denominado por el propio Seilacher como el "factor descuidado" (neglected factor) (Briggs 2014). A pesar de haber transcurrido tres décadas desde la publicación de Seilacher y colaboradores (1985) en dónde se planteaba la importancia de los tapetes microbianos en la formación de los Konservat-Lagerstätten, han aparecido muy pocos trabajos tafonómicos experimentales con estas comunidades. La inherente complejidad asociada al diseño de experimentos que incluyan tapetes probablemente sea una de las causas que expliquen esta carencia. Hay, no obstante, un número considerablemente mayor de trabajos empíricos llevados a cabo con "biopelículas" (biofilms) de microorganismos heterótrofos, comunidades mucho más simples, en donde se ha intentado determinar la implicación de la actividad microbiana en diferentes aspectos tafonómicos (e.g., Briggs y Kear 1993, Hof y Briggs 1997, Sagemann et al. 1999, Raff et al. 2008). ¿Pero, qué diferencia existe entre estos biofilms y los tapetes microbianos y cómo afectaría a su papel en la fosilización?

BACKGROUND

Fossil record is one of the most reliable sources to understand the evolution of Earth and its ancient ecosystems. Konservat-Lagerstätten are very valuable sites due to the exceptional preservation of the remains (Seilacher 1970). These deposits are a unique window to report the major events in evolution and biogeochemical variations through geologic history (Butterfield 2003). Conservation of the remains found in these Konservat-Lagerstätten is so extraordinary that even soft tissue, extremely labile material, has fossilized. This kind of preservation is a great challenge for taphonomy, the science in charge of describing and explaining fossilization. The origin of Konservat deposits has usually been linked to lacustrine or shallow-marine systems, or more scarcely to amber, permafrost, peat bog and tar sand deposits (Allison 1988). Adolf Seilacher, one of the most recognized experts in the field of the paleontology and taphonomy, devoted most of his carrier to try to unveil the secrets beyond the origin of these deposits. After a thorough analysis of the fossil record belonging to numerous Konservat, Seilacher et al. (1985) identified the presence of microbial mats among the three major factors for exceptional preservation. According to the authors, these microbial communities would promote the early diagenesis and notable conservation of the remains. That year, Jean-Claude Gall, studying several fossils and the surrounding sediment, also depicted the effect of "microbial veils" in the origin of some exceptional rests. However, the actual mechanism(s) to explain how microbial mats promote fossilization remains unknown. This lack of strong experimental evidences lead to Seilacher to designate this microbial factor as "neglected" (Briggs 2014). Three decades after the original paper by Seilacher et al. (1985) stating the role of mats in the genesis of several Konservat-Lagerstätten, only a few taphonomic experiments have tested the influence of microbial mats in preservation. This scientific gap can be partially explained by the inherent complexity of developing protocols to assay with microbial mats. Major approaches to theinfluence of microorganisms in the taphonomic process have been performed using heterotrophic biofilms as model community (e.g., Briggs y Kear 1993, Hof y Briggs 1997, Sagemann et al. 1999, Raff et al. 2008). However, this kind of biofilms is much more simple than actual microbial mat communities; therefore, are there any differences between microbial mats and biofilms that could help to understand the fossilization process?

TAPETES MICROBIANOS, UNA COMUNIDAD COMPLEJA

El término biofilm hace referencia a la forma de crecimiento de las comunidades microbianas en el medio natural. Tras la gran explosión de la Microbiología a finales del siglo XIX, con el aislamiento y proliferación de los microorganismos en el laboratorio, se asumía que su desarrollo en el medio ambiente era de tipo planctónico. Sin embargo, con el desarrollo de la Ecología Microbiana desde mediados del siglo XX, se reconoció que la forma habitual de crecimiento de los microorganismos en la naturaleza es en forma agregada, adheridos a superficies orgánicas o inorgánicas y en interfases (sólido/liquido, sólido/aire, líquido/aire, o entre de líquidos de distinta densidad), formando comunidades más o menos complejas, sobre diferentes sustratos y en una gran variedad de ambientes, a las que se denominó biofilms (biopelículas). Estas comunidades no son un simple agregado de células independientes, sino que crecen de forma integrada a nivel fisiológico rodeadas por una matriz mayoritariamente orgánica, constituyendo como un pseudo-organismo en el que sus integrantes modifican su expresión génica, pudiendo generar fenotipos completamente diferentes a los expresados durante un crecimiento aislado de sus poblaciones (Costerton 2012, Stolz 2000). El desarrollo de un biofilm es un proceso que requiere de gran coordinación, interacción y comunicación entre las diferentes poblaciones que lo conforman. Este complejo proceso puede dividirse en tres etapas: 1) atracción y adhesión a la superficie de los primeros colonizadores de la comunidad. Estas primeras células son las encargadas de generar una matriz extracelular fundamental para la agregación de partículas bióticas y abióticas, la protección frente a agentes externos (*i.e*. antibióticos), la retención de moléculas señalizadoras, etc. (Sauer et al. 2002); 2) división celular activa y crecimiento de la comunidad; y 3) maduración de la comunidad que implica la generación de una arquitectura organizada y más o menos compleja, con comunicación entre las diferentes poblaciones, así como una re-distribución de los nutrientes. Un suceso de especial relevancia para la formación del biofilm es el momento en que los organismos dejan de comportarse como una célula individualizada y pasan a expresar los genes y producir las sustancias correspondientes a un tipo de vida agregado. Este evento se conoce como *Quorum sensing*, y permite a la comunidad tener una noción de densidad poblacional. A medida que aumenta el número de células se va incrementando la concentración de diversos compuestos señalizadores conocidos como autoinductores. Así, cuando la cantidad de estas moléculas producidas alcanza un cierto umbral, las células embebidas en la matriz detectan dicha densidad poblacional y pasan a comportarse como una comunidad (para más información sobre este proceso, ver Ramsey et al. 2012).

En la naturaleza, la mayor parte de estos biofilms suelen ser finas películas, también conocidas como velos microbianos, de no más de 3-5 mm de grosor y con un bajo índice de diversidad poblacional. En ambientes iluminados (e.g. superficie de rocas sumergidas, charcas de lluvia, etc.) se pueden encontrar biofilms simples constituidos principalmente por microorganismos fotosintéticos, creciendo a expensas de la luz y los nutrientes

inorgánicos extraídos del medio. Pero allá donde haya materia orgánica (MO) disponible, los biofilms están dominados por microorganismos heterótrofos, desarrollándose sobre cualquier superficie que contenga MO, ya sea directamente sobre seres vivos o sobre cualquier resto orgánico (cadáveres, pellets fecales, sedimentos, etc.). Este es el tipo de biofilm presente en la mayoría de los experimentos tafonómicos publicados por diversos autores (ver apartado *Tafonomía Experimental* en este mismo capítulo). Ahora bien, en determinadas condiciones ambientales, las comunidades formadoras de biofilms pueden llegar a alcanzar un alto índice de diversidad poblacional, dando lugar a formaciones complejas, de grosores muy superiores, que presentan en su conjunto las características que definen un ecosistema: Un flujo interno de energía y un reciclado de nutrientes, llevados a cabo por las poblaciones de productores primarios (fotosínteticos, i.e. fotoautótrofos y/o quimiosintéticos, i.e. quimioautótrofos) y consumidores/descomponedores (heterótrofos) constituyentes de la comunidad, organizadas según diversos gradientes ambientales. Estas comunidades, que crecen en forma de biofilm complejo, y que se caracterizan por constituir un ecosistema en sí mismas (Stolz 2000), es lo que conocemos como **tapetes microbianos.**

En ambientes iluminados, el tapete comienza su formación a partir de la actividad de los microorganismos fotosintéticos a los cuales se asocian poblaciones heterótrofas y quimiosintéticas dando lugar a densas comunidades microbianas con una estructura organosedimentaria multilaminada que sigue un gradiente lumínico y químico (Cohen y Rosenberg 1989, Wierzchos et al. 1996). Estas capas se encuentran en constante crecimiento. En aquellos tapetes dominados por cianobacterias fotosintéticas oxigénicas, éstas se sitúan en superficie, asociadas con microorganismos heterótrofos aerobios. Esta capa superficial se dispone sobre láminas de fotótrofos anaeróbicos anoxigénicos mezclados con otros microorganismos anaerobios, heterótrofos o quimiosintéticos, que van siendo dominantes según se extingue la luz y disminuye el potencial redox (Eh) en las capas profundas. La transición entre la zona óxica a la anóxica se lleva a cabo en unos pocos milímetros (Fig. 1.1). En el interior de los tapetes se establece una íntima relación tanto entre individuos como entre poblaciones debido a la existencia de una densa matriz extracelular compuesta principalmente por diversos polisacáridos (EPS, de sus siglas en inglés), lípidos y ácidos nucléicos que se encuentran anclados, junto con las células, a un andamio de glicoproteínas como fibronectina, integrinas o colágeno (Decho 1990). La matriz es responsable de la cohesión de la comunidad y de su unión al sustrato al mismo tiempo que promueve la interacción entre los ciclos biogeoquímicos haciéndolos muy dinámicos (de Beer y Kühl 2001). Los productos resultantes de los diferentes procesos metabólicos pueden ser incorporados por los EPS (Lock 1993), evitando así la pérdida de nutrientes. Esta matriz tiene el potencial de restringir el acceso de algunos agentes antimicrobianos como antibióticos (Sauer et al. 2002) y ofrece protección contra la radiación UV, cambios bruscos de pH, presión osmótica y contra la desecación (de Beer y Kühl 2001). Así mismo, se ha comprobado que la matriz de EPS secuestra metales, iones y toxinas (Decho 1990, Flemming 1993, Wolfaardt et al. 1998).

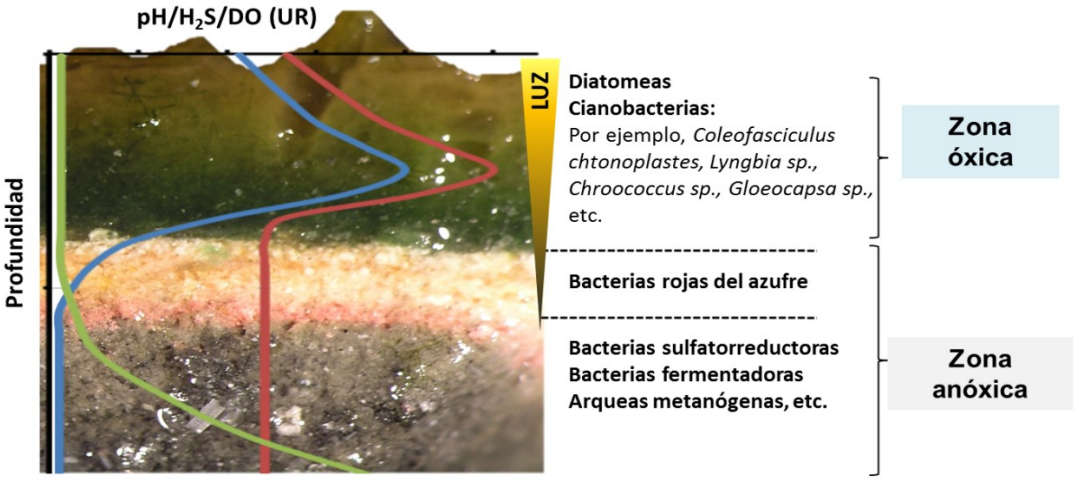


Fig. 1.1: Perfil de un tapete microbiano mostrando las diferentes capas que lo conforman. A la derecha se detallan algunas de las poblaciones que se pueden encontrar en cada zona. Se muestran también las variaciones en pH (rojo), DO (azul) y H_2S (verde) en función de la profundidad. UR: unidades relativas. Aunque el grosor depende de la comunidad, el espesor de la capa activa de un tapete microbiano (que se corresponde con lo que se muestra en la imagen) puede variar entre unos milímetros y algo más de un centímetro.

En la actualidad, los tapetes microbianos fotótrofos se encuentran preferentemente en ambientes extremos tales como ambientes hipersalinos (continentales o marinos), manantiales termales y algunos lagos Antárticos. Sin embargo, en el pasado se desarrollaron de forma globalizada en los mares Precámbricos, durante la época conocida como la "Era de los Estromatolitos", hace entre 2.500 y 550 Ma. La posterior aparición de los metazoos (Cohen 1989) pudo limitar drásticamente su distribución debido a la actividad ramoneadora. Los estromatolitos son rocas sedimentarias carbonatadas y de estructura laminar creadas por la actividad de los microorganismos que forman tapetes microbianos. El crecimiento de los estromatolitos se sustenta en la capacidad de dichos tapetes de captar, unir y cementar gran cantidad de partículas del sedimento (Krumbein 1979), sumado a la precipitación bioinducida de minerales (Decho y Kawaguchi 2003, Dupraz et al. 2009), en dónde las capas superficiales se mantienen en crecimiento activo. Sin embargo, no todos los tapetes microbianos tienen la capacidad de litificar. Con frecuencia, estas comunidades, aun cuando mantienen cierta capacidad cementante y de bioprecipitación, no llegan a formar estromatolitos.

Tabla 1.1: Resumen de la influencia de los principales metabolismos presentes en el tapete microbiano en la formación (+) o disolución (-) de carbonatos, yesos o fosfatos (como por ejemplo, fosfato cálcico) (Thompson y Ferris 1990; Dupraz et al. 2009; Dupraz y Visscher 2005; Visscher y Stolz 2005; Saghaï et al. 2015).

Proceso Metabólico	Carbonatos	Yesos	Fosfatos
Fotosíntesis oxigénica	+	+	-
Fotosíntesis anoxigénica	+	+	-
Sulfatorreducción	+	-	+
Sulfooxidación	-	+	-
Fermentación	-	-	+
Respiración aerobia	-	-	+

Los tapetes microbianos, por tanto, forman parte de la historia de la Tierra desde etapas muy tempranas (Schopf 2006). Su presencia ha influido enormemente en la evolución del planeta (Des Marais 2003) y, por ello, entender la actividad de estas

comunidades, en especial la mineralización mediada por microorganismos, podría ayudar a la interpretación posterior del registro fósil. De hecho, los tapetes microbianos actuales inducen la formación de un amplio rango de precipitados como resultado de la interrelación de las actividades biológicas y de las condiciones ambientales (Spadafora et al. 2010). La formación de los estromatolitos, por ejemplo, resulta del crecimiento de la comunidad microbiana y de la progresiva captación y precipitación de partículas minerales (Dupraz et al. 2006). Mediante su metabolismo (Fig. 1.1) los microorganismos modifican el ambiente geoquímico incidiendo, por lo tanto, en las condiciones para la precipitación o disolución de minerales (Tabla 1.1) (Dupraz et al. 2009). En el caso de los carbonatos, por ejemplo, actividades como la fotosíntesis oxigénica o la reducción del sulfato estarían favoreciendo su formación debido al incremento localizado del pH mientras que, por el contrario, otros procesos como la respiración aerobia, la sulfooxidación o la fermentación conducirían a una bajada del pH circundante y, por consiguiente, a su disolución (Visscher y Stolz 2005). De hecho, recientes estudios que emplean técnicas moleculares, como metagenómica, confirman que algunas cianobacterias así como bacterias sulfatorreductoras y ciertos linajes de fotosintéticos anoxigénicos poseen genes que participan en las rutas de bioprecipitación, pudiendo intervenir potencialmente en los procesos de formación de carbonatos (Saghaï et al. 2015). En ocasiones, el metabolismo es incluso responsable de la presencia diferencial de fases minerales. Así, por ejemplo, la actividad de las bacterias sulfatorreductoras, además de favorecer la formación de carbonatos, disolvería los posibles yesos del ambiente mediante el ataque a sus grupos sulfato si éste fuera limitante, pero si la concentración local de sulfato es elevada (fruto de la sulfooxidación, por ejemplo), se favorecería en cambio la precipitación de yesos (Thompson y Ferris 1990). Además, la propia estructura y composición del tapete microbiano parece participar en el proceso de biomineralización. La matriz de EPS es un punto activo para la nucleación y formación de minerales, determinando tanto la morfología como la mineralogía de sus fases (Braissant et al. 2003). Además, los EPS, que se encuentran cargados negativamente, poseen una gran capacidad para captar y unir grandes cantidades de cationes mono o divalentes (Sutherland 2001), inhibiendo por lo tanto su precipitación debido a que son retirados del entorno. De hecho, la matriz y la pared de las células son un punto de especial importancia en la unión y captación de hierro (Kornhauser y Ferris 1996). Este mecanismo de secuestro debe ser por lo tanto reducido o impedido para que se favorezca la precipitación mineral, y una de las vías sería la degradación biológica de los EPS (Dupraz et al. 2009). De hecho, la matriz no sólo sirve como andamiaje para la comunidad sino que se ha comprobado que también es una fuente de carbono orgánico para la obtención de energía (Paerl et al. 2001). La degradación de la porción bio-disponible de EPS supondría la liberación localizada de los iones secuestrados por los polímeros, favoreciendo la precipitación de minerales ferrosos (Kornhauser y Ferris 1996) o carbonatos (si se liberara calcio - Dupraz y Visscher 2005). En este caso, los restos de la matriz actuarían además como centro de nucleación (Lowenstam 1981), permitiendo la cristalización de calcita o yeso por ejemplo (Thompson y Ferris 1990). Este es un punto clave para la bioprecipitación, ya que se ha comprobado que en ocasiones, aunque la solución se encuentre sobresaturada, la floculación y posterior precipitación mineral se ve impedida por la ausencia de un punto adecuado de

nucleación, algo especialmente habitual en el caso de los silicatos (Kornhauser y Ferris 1996). Sin embargo, los mecanismos de nucleación y crecimiento de las estructuras minerales, así como la definición de la textura final, asociados a la actividad microbiana, están aún por concretar (Spadafora et al. 2010).

PALEONTOLOGÍA Y TAPETES MICROBIANOS

El estudio del registro fósil es una tarea compleja debido al sesgo existente en los fósiles preservados ya que se encuentra dominado principalmente por restos de organismos marinos con valvas (Allison y Briggs 1993). Debido a la dificultad de los tejidos blandos para fosilizar sólo en un número reducido de ocasiones se han obtenido fósiles con tejido no mineralizado (es decir, diferente del esqueleto interno o externo, dientes u otras estructuras duras) preservado (Sansom et al. 2010). Y ello, sin tener en cuenta el uso restrictivo que algunos autores hacen del término tejidos blandos, entendiendo como tal sólo aquellos que son fácilmente degradables (lo que excluye estructuras recalcitrantes como la exocutícula de los insectos) (Briggs 1995). Sin embargo, existen una serie de yacimientos, llamados Konservat-Lagerstätten o depósitos de conservación, en los que son comunes los restos de fósiles excepcionalmente preservados, incluso con tejidos blandos a pesar de su elevada facilidad para ser descompuestos. En el pasado, el estudio de estos fósiles excepcionales no fue tenido en cuenta en muchos análisis de los patrones de evolución ya que se consideraban anomalías que introducirían aún un mayor sesgo en el estudio (Allison y Briggs 1993) al tratarse de eventos de evolución no comunes (Conway Morris 1985). Recientemente, tras comprobarse que la preservación de tejidos blandos es más frecuente de lo que se esperaba (Allison y Briggs 1991), los Konservat-Lagerstätten han pasado a ser de vital importancia en paleontología ya que proveen de una información mucho más completa y fiable de las comunidades antiguas que los yacimientos ordinarios, permitiendo incluso estudios extensos de taxonomía, como en el caso de los anuros de Las Hoyas (Báez 2013).

Son numerosos los autores que dentro del ámbito de la Paleontología han teorizado sobre la posible implicación de los tapetes microbianos en los procesos de fosilización. Su aparición en diversos yacimientos es relativamente frecuente, especialmente en el caso de los Konservat-Lagerstätten, habiéndose identificado en depósitos que datan desde el Cámbrico Inferior (542-510 Ma) hasta el Mioceno Superior (7,2-5,3 Ma) (Tabla 1.2). La presencia de tapetes microbianos se ha determinado generalmente en base a evidencias indirectas, *i.e.*, perfiles sedimentológicos, existencia de células dispersas, estructuras sedimentarias asociadas a microorganismos (MISS, del inglés “Microbially Induced Sedimentary Structures” [Gerdes et al. 1993, Hagadorn y Bottjer 1997, Noffke 2009]), pero son raros los depósitos en los que se pueden observar directamente estas comunidades microbianas fosilizadas como en el caso del yacimiento de Riversleigh (Cenozoico Medio, Australia) (Arena 2008) o, en su defecto, microorganismos filamentosos con morfologías similares a ciertos tipos de cianobacterias que componen típicamente un tapete como en Solnhofen (Devónico Medio, Alemania) (Keupp 1977).

Tabla 1.2: Algunos depósitos de conservación de diferentes Periodos y Épocas con evidencias de posible presencia de tapetes microbianos.

Yacimiento	País	Periodo	Autores
Formación Meishucun	China	Cámbrico Inferior	Dornbos et al. 2004
Formación Wheeler	USA	Cámbrico Medio	Gaines et al. 2005
Amadeus Basin	Australia	Ordovícico	Young 1997
Formación Tonoloway	USA	Silúrico Superior	Vrazo et al. 2014
Solnhofen	Alemania	Devónico	Keupp 1977
Formación Tlayúa	México	Cretácico	Alvarado-Ortega et al. 2007
Las Hoyas	España	Cretácico Inferior	Buscalioni y Fregenal-Martínez 2010
Formación Agrio	Argentina	Cretácico Inferior	Fernández y Pazos 2013
Uña	España	Cretácico Inferior	Gomez et al. 2001
Formación Crato	Brasil	Cretácico Inferior	Martill et al. 2007
Hvar	Croacia	Cretácico Superior	Seilacher et al. 1985
Riversleigh	Australia	Oligoceno-Mioceno	Arena 2008
Rubielos de Mora	España	Mioceno Inferior	Peñalver-Mollá y Engel 2006
Libros	España	Mioceno Superior	McNamara et al. 2009

Con frecuencia, diversos ejemplares de algunos de estos yacimientos de diferentes épocas presentan una fina cubierta de origen microbiano, como en Las Hoyas (Cretácico Inferior, España) (Briggs et al. 1997, Gupta et al. 2008) o Nuspligen (Jurásico, Alemania)(Briggs et al. 2005). Basándose en la frecuencia de detección de tapetes microbianos en depósitos con fósiles bien conservados y en las características intrínsecas de este tipo de comunidad (ver apartado anterior), se ha venido postulando en las últimas décadas que los tapetes pudieran estar favoreciendo la fosilización (Gall et al. 1985). De hecho, Seilacher et al. (1985) establecieron que estos yacimientos tan excepcionales podían formarse por tres factores independientes pero no excluyentes (Fig. 1.2): el enterramiento rápido (obrution), la deposición en aguas estancadas (stagnation) y el denominado como sellado bacteriano, un concepto que intentaría sintetizar una serie de procesos llevados a cabo por los tapetes microbianos. El exhaustivo análisis del registro fósil asociado a estas comunidades (e.g. Gall 1990, Seilacher 1990, Behrensmeyer 2001) , además de reforzar esta hipótesis permitió a Briggs (2003), basándose en los planteamientos de Seilacher et al. (1985), concretar la participación de los tapetes en el proceso de fosilización en: 1) la protección del cadáver mediante el retraso en la descomposición; 2) un rápido enterramiento que evitaría la desarticulación del cuerpo y la acción de carroñeros o la erosión mediante las corrientes; 3) la generación de moldes e impresiones que copiarían en negativo la superficie de los cuerpos, 4) la compactación de los sedimentos y 5) la mineralización de los restos. Estos procesos (especialmente la formación de moldes y cementación del sedimento) permitirían explicar, por ejemplo, la aparición de numerosos fósiles que mantienen la estructura externa de la piel como en algunos dinosaurios (Platt y Hasiotis 2006, Kim et al. 2010) o determinadas estructuras anatómicas de tejido blando, como la bolsa gular o

la cresta del terópodo *Pelecanimimus* (Briggs et al. 1997). Estos mecanismos pudieran también ser relevantes en el marco de la antropología, por ejemplo, preservando huellas de homínidos primitivos (Marty et al. 2009), aportando así una información de enorme valor para la investigación concerniente a las poblaciones humanas del pasado.

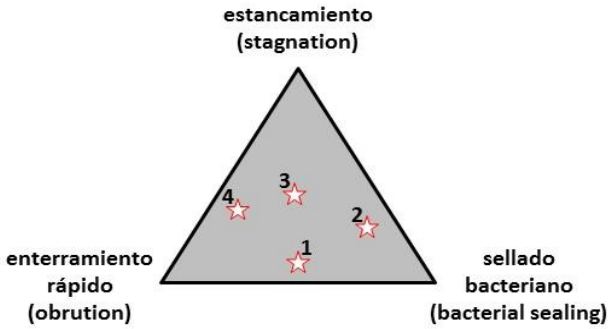


Fig. 1.2: Triángulo de preservación propuesto por Seilacher et al. (1985) en el que se sintetiza la teórica repercusión que ejercieron cada uno de los tres factores de preservación expuestos (enterramiento rápido, estancamiento y sellado bacteriano) en la formación de los diferentes Konservat-Lagerstätten. Como ejemplo, los yacimientos que presentan fauna de Ediacara (Proterozoico; 1) y el yacimiento de Hvar (Cretácico Superior, Croacia; 2), podrían ser explicados preferencialmente por la actividad del sellado bacteriano en conjunción con el enterramiento rápido o el estancamiento, respectivamente. En el caso de Solnhofen (Devónico Medio, Alemania; 3) los tres factores tendrían una gran participación mientras que la formación de los fósiles encontrados en Bundenbach (Jurásico Superior, Alemania; 4) se debería principalmente al estancamiento en agua con poco oxígeno y un rápido enterramiento.

De acuerdo con el triángulo de preservación de Seilacher et al. (1985) (ver Fig. 1.2) y otros estudios posteriores (e.g. Gehling 1999 o Narbonne 2005), los yacimientos que presentan fauna de Ediacara son uno de los casos más paradigmáticos de la conservación mediante tapetes. Se denomina fauna de Ediacara (o fauna vendiense) a una serie de organismos primitivos, de cuerpo blando, sésiles, que tenían forma tubular o de hoja. Estos organismos surgieron después de la explosión de Avalón (Shen et al. 2008) y poblaron la tierra en el periodo Ediacariense (Neo-Proterozoico, hace entre 635 y 542 Ma). Reciben este nombre debido a que los primeros restos fueron encontrados en el yacimiento de Ediacara, en Adelaida, Australia. Estos organismos, anteriores a la explosión del Cámbrico en la que surgen los grupos animales actuales, suponen los primeros seres pluricelulares complejos (Seilacher 1992) aunque desaparecieron posteriormente. De hecho, algunos autores se refieren a este periodo como un "experimento fallido" de la evolución, ya que esta fauna pudo incluso representar nuevos reinos eucarióticos desconocidos (Narbonne 2005). Los cuerpos de estos organismos no fosilizaron pero se preservaron como "máscaras de muerte", un relieve negativo o impresión que mantiene la forma y el contorno del cuerpo así como diversas estructuras superficiales (Gehling 1999) (Fig. 1.3). El estudio pormenorizado de dichos fósiles mostró además la existencia de láminas de carbonato con un posible origen biológico (Narbonne 1998, 2005), así como evidencias de ramoneo resultado de la actividad de organismos herbívoros que se alimentaban de los tapetes (Seilacher 1999).

Steiner y Reitner (2001) consiguieron incluso identificar filamentos que podrían corresponderse con cianobacterias. Para la formación de esta huella, Laflamme et al. (2011) postularon que los tapetes llevarían a cabo un mecanismo similar al que conduce a la formación de los MISS. Durante el crecimiento y desarrollo de la comunidad microbiana, los sedimentos quedarían estabilizados y compactados (Hagadorn y Bottjer 1997), permitiendo el mantenimiento de estructuras como arrugas (para más información acerca de los MISS, su clasificación y formación, ver Noffke et al. 2001).

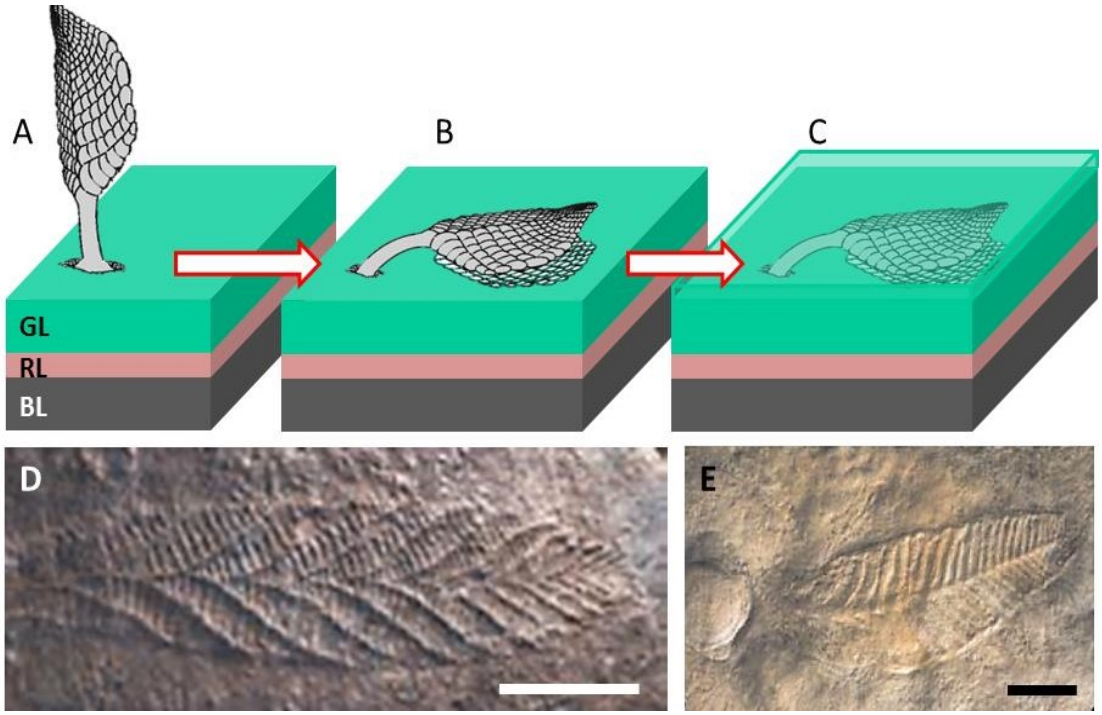


Fig. 1.3: Ejemplo de preservación de un organismo de la fauna de Ediacara (**A**). El organismo, al perder la posición vertical, queda apoyado sobre el tapete, que comenzaría a moldear e imitar la superficie del organismo (**B**). Posteriormente, la comunidad microbiana continúa su crecimiento, formado una cubierta protectora sobre el ejemplar, favoreciendo su fosilización (**C**). (**D**) se muestra un ejemplo de fósil de Ediacara: *Charniodiscus* sp. (South Australia Museum, P19690). (**E**) *Charnia masoni* encontrado en Charnwood, Inglaterra (ejemplar del Museo New Walk, Leicester). GL: Green Layer, capa superficial del tapete, compuesta principalmente por cianobacterias y otros organismos fotótrofos oxigénicos; RL: Red Layer, capa compuesta por poblaciones de fotótrofos anoxigénicos como bacterias rojas del azufre (de la familia Chromatiaceae); BL: Black Layer, con organismos heterótrofos o quimioautótrofos anaerobios. Figura modificada de McIlroy et al. (2009). Imágenes sacadas de Narbonne (2005). Las barras de escala representan 5 cm.

TAFONOMÍA EXPERIMENTAL

El importante papel atribuido a los tapetes microbianos en los procesos de preservación precisa de una comprobación experimental. La disciplina que estudia los procesos de fosilización y la formación de depósitos fosilíferos es una rama de la Paleontología conocida como Tafonomía. Los ensayos diseñados para esclarecer la implicación de los tapetes microbianos en la preservación se enmarcan pues dentro del ámbito de la Tafonomía, concretamente de la tafonomía experimental. La fosilización no es un proceso lineal con respecto al tiempo. La mayoría de las variaciones que se observan en los cuerpos muertos (cambios anatómicos, pérdida de peso o de volumen, etc.) ocurren rápidamente, por lo que es de suponer que las primeras etapas del proceso suceden también de forma rápida, produciéndose después pequeños cambios a muy largo plazo (Sansom 2014). A este respecto, la experimentación tafonómica utilizando diferentes comunidades microbianas, incluidas las de los tapetes, puede permitir diferenciar y acotar las etapas tempranas de fosilización, así como detectar la posible existencia de distintos procesos tafonómicos según las comunidades y variables ambientales utilizadas. Sin embargo, los microorganismos, aun cuando pueden favorecer la conservación y mineralización de los restos, son también los principales responsables de la descomposición de los cadáveres y resulta por lo tanto fundamental, discriminar los procesos microbianos que conducen a uno u otro fenómeno (mineralización o descomposición). Para ello, es de gran importancia una correcta descripción de los métodos usados y del origen y tipo de poblaciones microbianas empleadas. La aproximación más habitual, y que simula procesos de descomposición/fosilización sobre sedimentos en medio acuático, consiste en añadir al medio en el que se realiza el estudio, un inóculo microbiano proveniente de un sedimento normalmente de estuario o marino que da lugar a la formación de biofilms microbianos heterótrofos (e.g. Briggs y Kear 1993, 1994, Wilby et al. 1996, Hof y Briggs 1997, Martin et al. 2003, Martin et al. 2005). A lo largo de estos ensayos, que emplearon como animales de experimentación diversos crustáceos (huevos o adultos), se estableció que para que la mineralización de los tejidos blandos fuese posible era necesario bajo pH, anoxia y ambiente reductor, condiciones físico-químicas generadas durante la descomposición progresiva de los cuerpos, medidas mediante la utilización de microelectrodos, (Sagemann et al. 1999). Aunque generalmente los velos que crecen en estos experimentos son exclusivamente heterótrofos, en ocasiones se ha descrito la aparición de biofilms fotosintéticos, por ejemplo al adicionar aguas eutróficas provenientes de arrozales, que recubrían a los ejemplares en flotación lo que favorecería su hundimiento y preservación (Peñalver 2002). Por otro lado, muchos fósiles que contienen tejido blando conservado se encuentran fosfatizados (e.g. Martill 1988, 1990; Wilby y Briggs 1997; Arena 2008 o Gutiérrez-Marco y García-Bellido 2015) o, menos frecuentemente, piritizados (*e.g*. Bartels et al. 1998, Farrell et al. 2009; Botting et al. 2011). Varios trabajos tafonómicos experimentales han intentado saber cómo la actividad microbiana influye en este tipo de preservación así como bajo qué circunstancias ambientales ocurre. Briggs y Kear (1993, 1994), utilizando camarones (*Crangon crangon*) y Hof y Briggs (1997) usando gambas mantis (*Neogonodactylus*

oerstedii), observaron el reemplazamiento de algunos tejidos blandos internos (músculos) por fosfato cálcico ($CaPO_4$). En estos casos la actividad descomponedora de los microorganismos libera Ca y PO_4^{2-} a partir de las cutículas y baja el pH favoreciendo esta precipitación en detrimento de la de carbonato cálcico (Wilby et al. 1996). Posteriormente, Martin et al. (2003) observaron experimentalmente que la formación tanto de carbonato como de fosfato cálcico sobre huevos de langosta era independiente del pH del medio, por lo que propusieron que el factor clave para determinar la vía de mineralización no es el pH sino la composición química del ambiente. Así, la liberación de fosfatos durante la degradación microbiana de los tejidos y el consecuente incremento de su concentración en el medio determinaría la precipitación diferencial de carbonato o fosfato cálcico. Los experimentos para determinar cómo pudo ocurrir la piritización son muy escasos. No obstante, Brock et al. (2006) llevaron a cabo ensayos con ramitas de plátano de sombra (*Platanus acerifolia*) observándose la piritización sólo de algunas ramas. Aunque no lograron encontrar la causa que provoca la precipitación de pirita, sugirieron varias razones para explicarlo como, por ejemplo, posibles diferencias en la composición celular de las ramas o en las características de los sedimentos usados como cantidad de materia orgánica, composición geoquímica o tipo de poblaciones microbianas.

Es indudable, como se ha ido mostrando en este apartado, la importancia de la actividad microbiana en los procesos de fosilización, si bien aún estamos lejos de conocer los mecanismos biológicos implicados más relevantes. Algunos artículos, como los de Raff et al. (2008) y Raff et al. (2013) intentan aproximarse a este conocimiento. En estos trabajos se utilizaron inóculos de sedimento sobre embriones de erizo marino y se observó la progresiva sustitución de los tejidos propios del embrión por bacterias que mantenían la forma original, generando réplicas de los embriones. Por otro lado, Darroch et al (2012) intentaron esclarecer la influencia del tipo de poblaciones microbianas que intervienen en la preservación de las muestras mediante la secuenciación del gen 16S rRNA. La comparación de secuencias permitió identificar los filotipos bacterianos presentes en el tapete durante la formación de los moldes de larva. Esta información, aunque interesante, no es fácilmente extrapolable a otras situaciones. En términos de los procesos desempeñados por la comunidad microbiana las poblaciones presentes (i.e., taxones) tienen menor relevancia que su funcionalidad o metabolismo. Un mismo taxón podría desempeñar diferentes funciones y, por lo tanto, afectar a la descomposición/preservación de muy diferentes maneras, así como distintas poblaciones podrían desempeñar el mismo papel funcional. Para poder comprender cómo los microorganismos participan en la preservación serían más útiles otros tipos de aproximaciones moleculares como la transcriptómica, en la que se estudian los RNA mensajeros que se están expresando en la comunidad en un momento concreto (y por extensión, los genes que se encuentran activos), o la proteómica, que estudiaría las proteínas que están presentes. Esta información, directamente relacionada con los procesos metabólicos, permitiría conocer qué actividades microbianas están implicadas en la preservación de los cuerpos.

Otra aproximación, diferente a la incorporación de inóculos provenientes de sedimento, es el empleo de tapetes microbianos. Estas complejas comunidades pueden ser utilizadas directamente desde muestras naturales (Darroch et al. 2012) o desarrollados en laboratorio a partir de muestras naturales homogeneizadas (Guerrero et al. in press, Iniesto et al. 2013, 2015a,b). A pesar de la relativa escasez de experimentación con estos ecosistemas microbianos en comparación con los inóculos de sedimentos, se ha podido demostrar que los tapetes explicarían la creación de las "máscaras de muerte" (Darroch et al. 2012) responsables de la formación de fósiles como los de la fauna de Ediacara. En este experimento Darroch et al. (2012) depositaron larvas de artrópodo sobre una muestra natural de tapete microbiano procedente de North Branford (Connecticut, USA). Para favorecer la observación posterior, el cadáver y la comunidad microbiana fueron separadas por una fina lámina de plástico sobre la que se depositó sedimento y el conjunto se dejó evolucionar durante 6 semanas a temperatura ambiente. A pesar de que la presencia de la lámina de plástico altera el crecimiento normal del tapete, limitando, por ejemplo, la incidencia de luz al recubrirlo de sedimento, los resultados mostraron que en presencia de una base de tapete, las impresiones en negativo del cuerpo en su superficie se mantenían durante más tiempo y con una mayor fidelidad que en los controles, en donde la larva se había depositado directamente sobre sedimento. Además se pudo observar la aparición de precipitados, posiblemente de pirita.

Como ha quedado expuesto, la tafonomía experimental ha crecido en los últimos años, especialmente en lo que se refiere a la implicación de los microorganismos, realizándose importantes avances en la materia. A pesar de ello, la influencia de la presencia de tapetes microbianos en las etapas tempranas de fosilización mantiene numerosos aspectos aún por desvelar. Determinar el impacto real de estas comunidades en la formación de fósiles es esencial para poder explicar y comprender el registro fósil y, por lo tanto, describir los ecosistemas del pasado.

OBJETIVOS Y ESTRUCTURA DE LA TESIS

El objetivo general de la presente tesis ha sido aproximarse, a partir de la tafonomía experimental con tapetes microbianos, al conocimiento de los procesos y mecanismos mediante los cuales los cadáveres se preservan y transforman en fósiles.

Para comprender los procesos que explican la formación de fósiles excepcionalmente preservados, especialmente en el caso de tejidos blandos, se han descrito dos posibles vías de preservación (Briggs 2003): 1) la preservación orgánica y 2) la mineralización mediada por los microorganismos. La preservación orgánica es el mantenimiento prolongado de las biomoléculas originales del organismo (como lípidos o proteínas) que se irán transformando en otras durante el proceso de fosilización. Esto sucede cuando la descomposición se ve muy ralentizada o incluso impedida y puede verse favorecida o modificada por el proceso diagenético. Se ha comprobado que durante la diagénesis la materia orgánica preservada puede verse sometida a altas presiones y temperaturas dando lugar, por ejemplo, a querógeno, que posteriormente se transformaría en filosilicatos como los encontrados en los fósiles de Burgess Shale (Cámbrico Medio, Canadá) (Page et al. 2008). Un factor clave para este tipo de preservación, como se ha dicho, es el retraso en la descomposición. Por lo tanto, uno de los sub-objetivos de esta tesis ha sido **comprobar si los tapetes microbianos retrasaban este proceso con respecto al deterioro de los cuerpos colocados únicamente sobre sedimento** (Capítulos 2 y 3, Fig. 1.4). El retardo en la degradación de los restos orgánicos debe atribuirse a condiciones ambientales concretas que varios trabajos han intentado identificar mediante diversas aproximaciones experimentales (e.g., Allison 1990, Briggs y Kear 1993). Los autores encuentran que la anoxia, el bajo pH y un ambiente reductor son factores necesarios para la preservación orgánica. Sin embargo, estos trabajos fueron realizados con comunidades microbianas heterótrofas formadoras de biofilms simples. La utilización de tapetes microbianos fotosintéticos en la experimentación abre el espectro de condiciones ambientales que pueden estar implicadas en la preservación orgánica. Por consiguiente, otro de los sub-objetivos de la tesis ha sido **conocer los valores de las variables ambientales que pueden estar relacionadas con el fenómeno de la preservación en tapetes microbianos** (Capítulo 5, Fig. 1.4).

La segunda ruta de preservación, la mineralización mediada por microorganismos, ha sido generalmente presentada como opuesta a la anterior (Sansom 2014). Al contrario que la preservación orgánica, que requiere una ralentización de la descomposición, la mineralización autigénica dependería de la rápida acción de los microorganismos que descomponen los cadáveres. Éstos generarían las condiciones favorables para la formación de minerales preservando los tejidos aún no descompuestos y que quedarían, por ejemplo, fosfatizados (Briggs y Kear 1994), o piritizados (Brock et al. 2006). En este caso, la escala temporal se reduce considerablemente ya que para que sea posible la mineralización de tejidos ésta debe ser muy precoz, evitando así la total descomposición

de los cuerpos. Sin embargo, si la comunidad microbiana implicada en el proceso forma parte de un tapete microbiano fotótrofo, la presencia de una gran diversidad de poblaciones y lo que eso supone en términos de amplitud y variedad de capacidades funcionales y metabólicas, podría plantear un sistema novedoso en el que los anteriores resultados podrían no tener validez y en el que el tiempo de degradación se alargaría (ver Capítulos 2 y 3). En este marco, la actividad microbiana podría mineralizar los tejidos a más largo plazo. De este razonamiento surge otro sub-objetivo, que se centra en **comprobar la aparición de bioprecipitados en los cuerpos, localizar su emplazamiento y estudiar su composición** (Capítulo 6, Fig. 1.4).

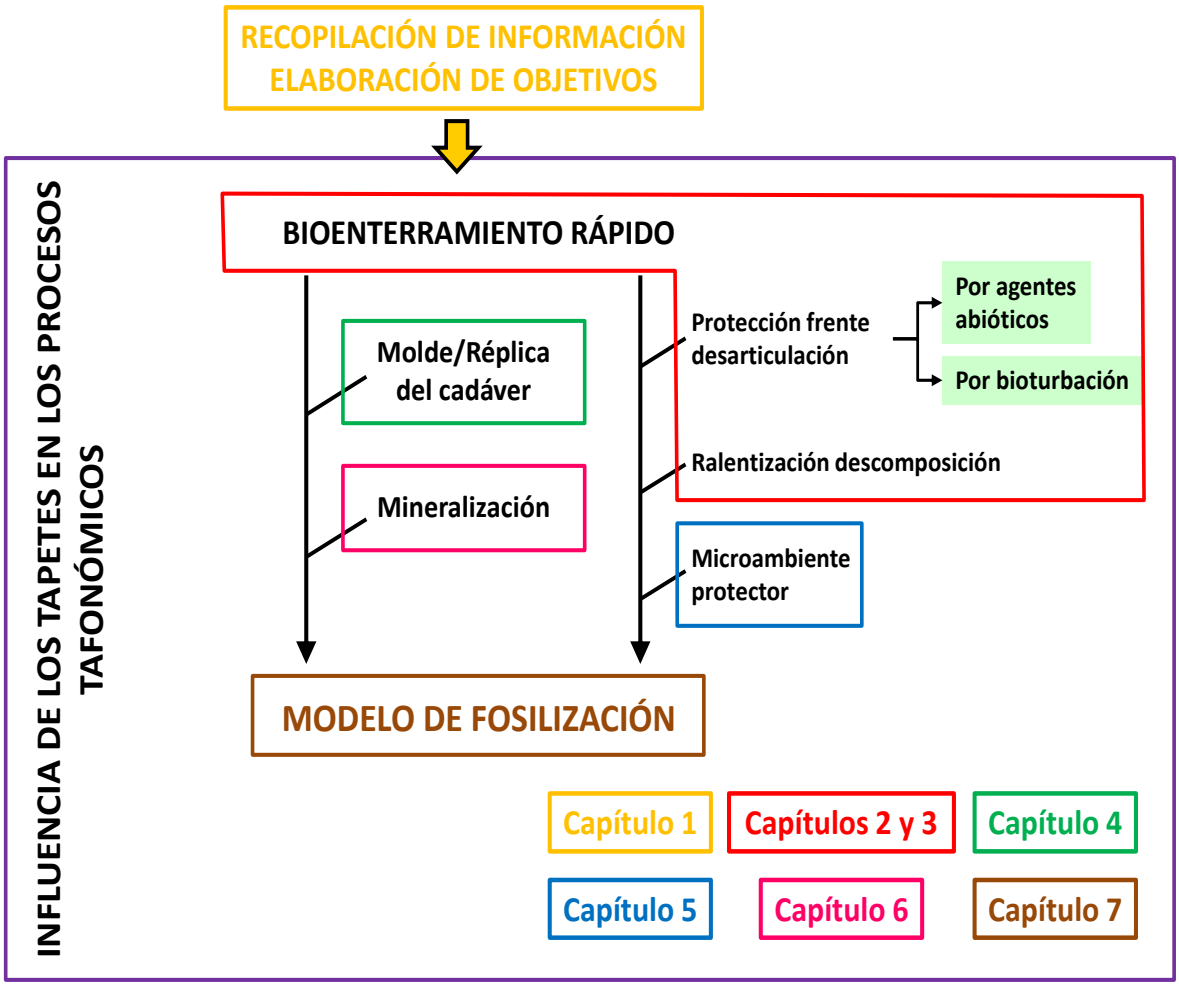


Figura 1.4: Resumen de la estructura de la tesis doctoral que muestra el esquema de trabajo, desde el planteamiento de las hipótesis previas en base a la información de la que se dispone (Capítulo 1), los experimentos realizados para poder dar soporte experimental a los postulados iniciales (Capítulos 2 a 6) y el modelo que se propone para explicar las etapas tempranas de la fosilización mediante tapetes microbianos (Capítulo 7).

Un fósil no tiene por qué ser únicamente el cuerpo de un organismo pretérito, son fósiles también cualquier vestigio de su existencia en la litosfera, por ejemplo sus huellas (icnitas), sus rastros (turbiditas), sus excrementos (coprolitos), la impresión de la superficie de su cuerpo o su contorno, etc. No es fácil encontrar trabajos tafonómicos en

donde se intente demostrar experimentalmente cómo pudieron formarse este tipo de fósiles, a excepción del de Darroch et al. (2012), en el cual se muestra que los tapetes microbianos podrían formar las "máscaras de muerte" (ver apartado 1.2 Paleontología y tapetes microbianos) y a que numerosos estudios, especialmente sobre fauna de Ediacara, habían recurrido como explicación a las impresiones fósiles de sus cuerpos blandos (Gehling 1999), o el de Marty et al. (2009), que trata de explicar la preservación de huellas de homínidos primitivos. La limitada información experimental que actualmente se tiene sobre cómo se formaron estos otros tipos de fósiles lleva a otro sub-objetivo de esta tesis: **intentar conocer el grado de fidelidad que los tapetes pueden lograr en la formación de impresiones y/o réplicas tanto de organismos de cuerpo blando (pequeños vertebrados) como duro (artrópodos)** (Capítulo 4, Fig. 1.4).

El conjunto de objetivos relatado a lo largo de esta sección pretende recoger y englobar los, hasta hoy, hipotéticos procesos de preservación mediados por tapetes microbianos que fueron planteados por Seilacher et al. (1985) y concretados por Briggs (2003). Basándose en la información de la que disponemos, la figura 1.4 muestra un esquema teórico preliminar de cómo la presencia de los tapetes favorecería la formación de fósiles. A partir de un proceso de cubrimiento (bioenterramiento), se activarían un conjunto de propiedades (*e.g.* retraso en la descomposición, formación de impresiones, bioprecipitación) que desembocarían en la posterior preservación de los cuerpos. Mediante la serie de experimentos que se expone en la presente tesis se busca, no sólo comprobar si los tapetes microbianos son un agente clave para la preservación de fósiles, sino también **comprender cómo se interrelacionarían cada uno de los procesos** (capítulo 7, Fig. 1.4). Los nuevos conocimientos obtenidos nos permitirán plantear, a modo de cierre, un modelo teórico de cómo se ven influenciadas las etapas tempranas de fosilización por la presencia de estas comunidades microbianas, revisando y ampliando el modelo procpuesto hasta el momento.

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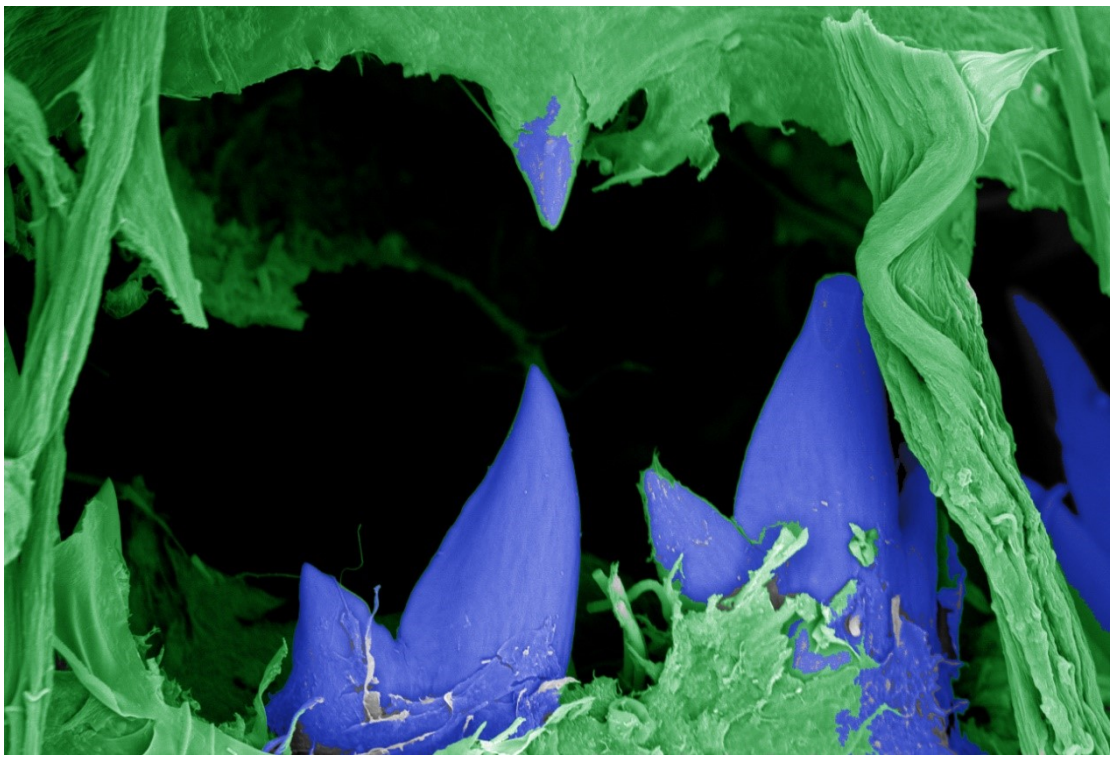
CAPÍTULO 2

INVOLVEMENT OF MICROBIAL MATS IN DELAYED DECAY: AN EXPERIMENTAL ESSAY ON FISH PRESERVATION



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ABSTRACT

Microbial mats have been implicated in exceptional fossil preservation. Few analyses have addressed how these complex-multilayered biofilms promote fossilization. The sequence of changes during decay of neon tetra fish were tracked up to 27 months, and their decomposition in mats was compared against nonmat sediments (control fish). Statistically significant differences in quantitative variables (length, width, and thickness) are provided (ANOVA test, in all cases, P < 0.001). Changes in the qualitative features (body-head, fins, scale connection, and eye and body coloration) were phenetically analyzed resulting in two clusters and highlighting that notable difference in decay began at day 15. Mat fish show a delayed decomposition maintaining the external and internal body integrity, in which soft organs were preserved after 27 months as shown by Magnetic Resonance Imaging. We discuss how the organization, structure, and activity of this community are interrelated, favoring exceptional preservation. Microbial mats entomb the fish from the earliest stages, forming a Ca-rich coat over the carcass while embedding it in an anoxic condition. This quick entombment provides important protection against abiotic and/or biotic agents.

Keywords: exceptional preservation, experimental taphonomy, fish decay, fossilization, microbial communities

RESUMEN

Los tapetes microbianos han sido implicados en los procesos de conservación excepcional, aunque sólo se han llevado a cabo unos pocos estudios para comprobar cómo estas complejas comunidades multilaminadas favorecen la fosilización. En este trabajo se ha realizado un seguimiento, a lo largo de 27 meses de la secuencia de cambios que tiene lugar durante la descomposición de peces neón tetra depositados sobre tapetes frente a peces control en sedimentos sin tapetes. El análisis estadístico de las variables morfométricas analizadas (longitud, anchura y grosor) demostró que las diferencias observadas eran significativas. Además, los parámetros cualitativos registrados (conexión cabeza-cuerpo, articulación de las aletas, estado de los ojos y coloración) permitieron el establecimiento, mediante un análisis de conglomerados jerárquicos, de grupos que separaban claramente los casos control de los ejemplares sobre el tapete, con diferencias que se incrementaban a partir del día 15. Los peces sobre el tapete mostraron un notable retraso en la descomposición, manteniendo la integridad interna y externa del cuerpo y conservando incluso tejidos blandos después de 27 meses, como se comprobó mediante resonancia magnética. Se discute como la interrelación de la organización, la estructura y la actividad de la comunidad microbiana favorece la preservación excepcional de los cuerpos. Los tapetes generan una envuelta que atrapa los cadáveres desde las primeras etapas creando inicialmente condiciones anóxicas y formando una cubierta rica en calcio. Este sarcófago confiere una notable protección frente a agentes bióticos y/o abióticos, manteniendo la integridad de los cuerpos.

Palabras clave: comunidades microbianas, descomposición de peces, fosilización, preservación excepcional, tafonomía experimental

INTRODUCTION

Microbial mats are complex benthonic multilayered communities. The community bears different physicochemical properties generated by light penetration and the activity of microorganisms along a vertical gradient (Cohen, 1989; Wierzchos et al., 1996). The multilayer mats grow continuously, with oxygenic photosynthetic (mainly filamentous cyanobacteria) and aerobic heterotrophic microorganisms in the oxic upper layer, overlying an anoxic deeper layer characterized by several anoxygenic photosynthetic bacteria (of the Chromatiaceae and Chlorobiaceae groups) and anaerobic microorganisms (such as fermenters or sulphate-reducing bacteria). The transition from the oxic to the anoxic zone and the flux and nutrient recycling among trophic levels occur within a few millimeters of the mat surface, giving these communities the character of a microecosystem in their own right.

During mat growth, organic remains, sedimentary particles, and bioinduced precipitates are trapped by the entangled structure of the community (Krumbein, 1979). In fact, microbial mats play a crucial role in the formation of microbially induced sedimentary structures (MISS) encompassing the regular deposition of sand, the accumulation of sediment, and the subsequent mat cover (Noffke, 2009). The frequent association of fossilized microbial mats with better-preserved fossils has been treated as a positive consequence for exceptional preservation, accumulation and ecological fidelity in Konservat Lagerstätten (Gall et al., 1985; Gall, 1990; Seilacher et al., 1985; Seilacher, 1990, 2009; Behrensmeyer, 2000; Briggs, 2003b; Buscalioni and Fregenal-Martínez, 2010). Mat properties that have been proposed to promote exceptional fossil preservation include: (1) envelopment (growth of the mat over the body); (2) pseudomorphism (replication of the body surface by microbial growth); (3) protection (from scavengers and from water currents) avoiding the breakup of the corpse; (4) delay in decay rate; and (5) bioprecipitation (Briggs, 2003a). The relevance of bioinduced precipitation (e.g., calcium carbonate) has been highlighted by several sedimentological and microbial ecological studies (Kühl et al., 2003; Decho and Kawaguchi, 2003; Dupraz and Visscher, 2005; Ludwig et al., 2005).

Accordingly, the preservative action of bacteria occurs during early biostratinomic phases, and actualistic approaches need to be designed to explore the role of biofilms in fossilization. Most experiments have used single-layered biofilms (e.g., experiments on Diptera, Peñalver, 2001; Echinoidea, Raff et al., 2008; and fish, Martín-Abad and Poyato-Ariza, 2010). Analyses testing complex, multilayered biofilms (i.e., microbial mats) are rare (e.g., Crustacea, Briggs and Kear, 1994; Sagemann et al., 1999). We present herein an actualistic approach, in which specimens of the Teleostei fish Paracheirodon inessi (family Characidae) were placed on biomats that were previously grown in the laboratory. Neon tetra fish were monitored up to 27 months, tracking the sequence of changes during decay. The aim of this experiment is to contrast the role of microbial mats in the initial stages of exceptional preservation, and to examine how the organization, structure, and activity of this community influence the factors involved in preservation, and how these factors are interrelated.

MATERIAL AND METHODS

Microbial mats used in the experiment were obtained from samples from La Salada de Chiprana (Zaragoza, Spain), which is a hypersaline (30‰–70‰), permanent, and shallow lake of endorheic origin in a semiarid region of the Ebro depression (Aragon, northeastern Spain). Salinity arises from the continuous arrival of mineralized water containing magnesium sulphate and sodium chloride, which are the main salts in this environment. Microbial mats, enhanced by the extreme physical and chemical conditions of the lake, cover the sediment from the shoreline to a water column depth of 1.5 m (Guerrero et al., 1991). Cohesion of the mat is conferred by the main filamentous cyanobacteria, *Coleofasciculus chtonoplastes* (formerly known as *Microcoleus chthonoplastes*).

The Chiprana mats were grown in the laboratory under controlled conditions. Glass tanks measuring 50 × 25 × 20 cm containing a 2–3 cm base of limestone overlain by a 3–4 cm layer of sediment from La Salada were used for mat development. Natural mat samples were ground up with a basic mixer (T25 IKA Labortechnik) and placed evenly over the sediment. Three of the tanks were illuminated by a 50 W halogen lamp (OSRAM Decostar 46870WFL). Incident illumination on the sediment surfaces was approximately 300 µmols m-2 s-1 (±1 µmols m-2 s-1) intensity. Tanks were exposed to a 10 h dark, 14 h light cycle. A fourth tank, without a mat, was kept in darkness as a control. Although it would be preferable to subject the control to the same diurnal cycle, the control chamber was kept in the dark to avoid mat growth from resistant phototrophic bacteria in the natural sediment. The mats were allowed to grow until they stabilized, exhibiting a structure and composition similar to those of natural mats. Conductivity (as an indirect measured of salinity), temperature, and pH were monitored in the course of the experiment (measured with a WTW-LF 330/SET conductivity meter and a 323 A WTW pH meter, respectively).Water conditions in the tanks were maintained at 27 ºC (±0.5 ºC), pH 7.9 (±0.2) and conductivity 57.1 mS cm-1 (±2.3 mS cm-1). Sterilized distilled water was added each day to maintain a 2-cm-deep layer of water and constant salinity

Paracheirodon inessi fish (neon tetra) were used as experimental animals. Specimens were analyzed (one from each microbial tank and two from the control tank) at 7, 15, 30, 45, 60, and 90 days. Additionally, several fish were kept longer in the mat to make subsequent observations possible. At days 120, 270, 540, and 810, fish from both kinds of tanks (with mat and control) were extracted to observe the entombment and decay state (see Long-Term Monitoring section). Specimens were chosen at random to maintain objectivity. *Paracheirodon inessi* was chosen on the basis of its morphological characteristics, which can be conveniently assessed during the decay process (e.g., large eyes that enable decay to be monitored, color to control the loss of brightness, scale organization) and size (2.3 ± 0.4 cm long). Following standard animal care protocol used at the Universidad Autónoma de Madrid, fish were asphyxiated with nitrogen and laid on the microbial mat or nonmat sediment, with a 2.5 cm gap between specimens to avoid possible interactions. This distance was established with preliminary experiments, in which there was no intersection of the sulphide halos between adjacent fish. A range

of quantitative and qualitative observations of these samples was made, according to the proposed objectives: (1) comparative measures of decay in the mat and control sediment samples; (2) the biostratinomic processes associated with entombment in complex mats; and (3) long-term monitoring of internal and external fish integrity.

Fish were manipulated by cutting a thin lamina of the mat and separating the carcass from the mat under the stereo microscope. For the control, fish were removed with a small spatula, in order to minimize damages, and cleaned with drops of water. Qualitative observations (see below Entombment and biostratinomic processes) were based on the changes in fragility of the decaying fish according to handling.

Comparative Decay Measure

As discussed above, fish were examined from each of the mat tanks and the control tank at set intervals. Decay was monitored to establish the sequence of decomposition, observing the following biostratinomic alterations: body-head connection, caudal and dorsal fin disarticulation, loss of coloration, scale organization, and eye decay (Fig. 2.1–2.2). The variables selected are based on analyses of tetrapod disarticulation sequences (Cambra-Moo and Buscalioni, 2003), wherein the biostratinomic alterations tracked the modular body organization (body-head; fins or appendages connections) rather than occurring randomly. Decompositional characteristics were scored as: 0, no changes; 1, light changes; 2, partial modification; 3, total modification. Data from mat and non-mat sediment samples were subjected to a Hierarchical Cluster Analysis using SPSS 15.0 for Windows. The results are represented as a dendrogram (Fig. 2.3).

The metric comparison was based on the length, width, and thickness of fish, making it possible to determine whether the mat has an influence on decay rate. These measurements were made with a 150 mm digital caliper. Measurements were taken before the animals were placed on the mat and on each of the aforementioned dates. The morphometric data were subjected to an Analysis of Variance (ANOVA) to determine whether the microbial mat was a significant factor in decay rate.

Early Entombment and Initial Biostratinomic Processes

To follow the envelopment process, the growth of microbial mat over fishes was monitored. Bodies were observed with the aid of an Olympus SZX ILLK200 binocular microscope and photographs were taken with an Olympus Camedia C5050 Zoom digital camera. Detailed observations were made of modifications of the eyes, fins, and scales using a Hitachi S-3000N Scanning Electronic Microscope (SEM). Samples were fixed with glutaraldehyde 2% (4 h), osmium tetroxide 1% (4 h) and dehydrated in an alcohol series of increasing concentrations (30%, 50%, 70%, 90%, 3 × 100%, 5 min each). Solutions were prepared with PBS (phosphate-buffered saline), and some residue remained after washing. Samples were dried at 37 ºC overnight. To facilitate SEM observations, the entire bodies were covered with gold to make electron conduction easier. During

observation under the SEM, bioprecipitates were localized and occasionally examined using Energy Dispersive X-ray analysis (EDX, with an INCA x-sight analyzer; Oxford Instruments). This makes atomic identification (and estimation of percentage of weight) of eventual superficial precipitates possible, thereby enabling the percentage of weight to be estimated. Carcasses were separated from the microbial cover to reveal the coating formed by the cyanobacteria and any mold that may have formed on the carcass, taking care not to disrupt the shape of the body. In four cases, longitudinal and sagittal sections were examined to observe and describe fish shape, the interface with the mat, and any possible changes on the mat and the surface of contact between the fish and its surroundings.

Long-Term Monitoring

After entombment in the mat, manipulation of the fish became difficult, making extraction impossible. New techniques, such as magnetic resonance imaging (MRI), were utilized to continue to observe taphonomic alteration of the specimens. MRI is a nondestructive technique that provides good contrast between the body parts and tissues. Two fish were taken from the mat after 270 days to analyse coverage. These fish and two additional fish at day 810 were removed from the tank with their intact microbial envelopment to evaluate morphological integrity. Integrity was studied using a Bruker BMT 47/40 MRI scanner (see Grimm et al., 2003; Riches et al., 2009 for the MRI technical application). MRI samples were compared with a control fish (9 months on nonmat sediments) and with a recently killed fresh fish. T2-weighted MRI was chosen to obtain the best possible contrast between the fish and the microbial mat. Contrast results from differential magnetic excitation of protons contained in water. Due to energy gained by excitation, higher hydration appears lighter in the image and less-hydrated tissue appears darker (Novelline, 2004).

RESULTS

Sequence of Changes During Decay

Monitoring the decomposition revealed important differences in the decay sequence between fish placed on the microbial mat and on nonmat sediment (Fig. 2.1). The variability in the decay sequence among specimens at each sample date was very low. Control fish showed advanced decay from the second week. At day 15, the exposed side of the fish exhibited substantial damage, the eye presents moderate decomposition (Fig. 2.1B), and the fins are considerably disarticulated (Fig. 2.1D). Subsequently, the decomposition of the fish became so advanced (Fig. 2.1F, day 30) that during manipulation for analysis the head became disconnected from the body and the carcass was reduced to several fragmentary remains, such as isolated bones from the spinal cord. In contrast to the control, fish from mat tanks exhibited only minor damages. Between days 7 and 30, the structural integrity of the fish did not alter. The fins and abdomen were trapped by cyanobacterial filaments (Fig. 2.1A) and by day 15, filaments had begun to grow over the whole body (Fig. 2.1C). In these initial days, several fish (30%) exhibited a hole in their abdomen, due to the production of gas by intestinal bacteria during the bloating phase. At day 30 the fish were almost totally covered by microbes (Fig. 2.1E). After this day, fish on the mat showed only slight changes in some structures, such as the fins, and color.

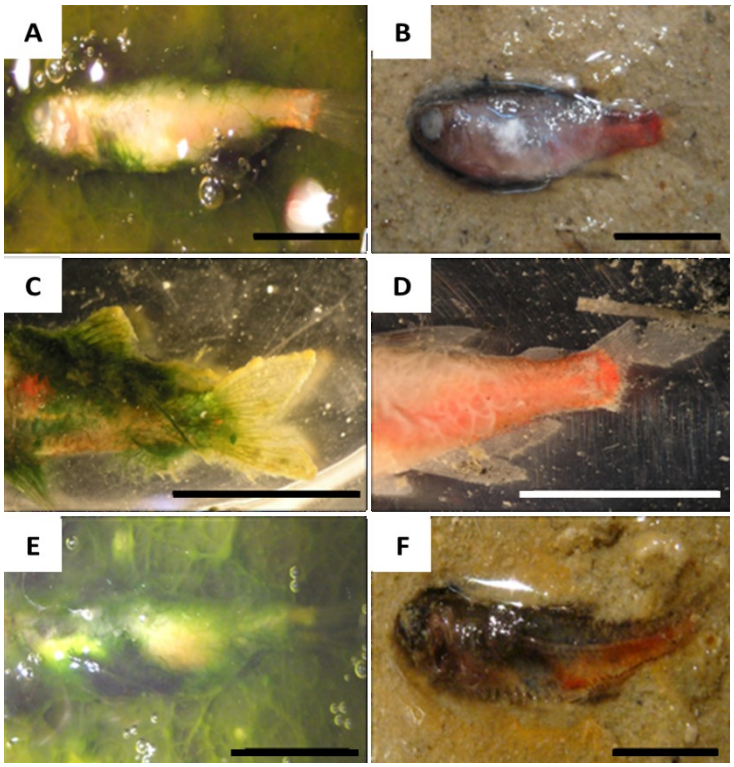

Fig.2.1: Sequence of changes caused by decay when fish is laid on microbial mat or on sediment (controls). (**A**) Fish on microbial mat on day 15. (**B**) Fish control on sediment on day 15 (note that the sediment below has turned black because of anoxic conditions caused by decay) (**C**) on day 15, detail of caudal fin partially covered by mat, completely articulated. (**D**) State of decay of caudal fin of fish control on day 15. (**E**) Fish covered by mat on day 30, retaining initial morphology. (**F**) Fish on sediment after 30 days. (Scale bar represents 0.5 cm).

Scanning Electronic Microscopy (SEM) was used to detail the biostratinomic changes of the eyes, fins, and scales between fish on mat and nonmat sediment (Figs. 2.2A–D). The upper eyeball of the fish on the mat (Fig. 2.2A) was complete, whereas that of the

fish from the control tank was substantially deteriorated (Fig. 2.2B). Dorsal fins also differed. Whereas the fins from control fish were rapidly disarticulated and the fin rays were fractured (Fig. 2.2D), the microbial mat fish maintained imbricated scales and articulated fins devoid of broken elements (Fig. 2.2C). Over time the differences became more pronounced. By day 30, the microbial mat fish retained their integrity with articulated scales and no pierced surfaces or holes (Fig. 2.2E), whereas the scales of the control fish were disarticulated and exhibited many holes (Fig. 2.2F). On day 90 the microbial mat specimens still maintained organized scales and tegument (Fig. 2.2G), while the control specimens were decomposed, with only a few fragmentary and unconnected remains (Fig. 2.2H). At this point further observation of the remains was of no comparative value. The integrity observed in fish extracted from the mat after this time revealed that their articulation was not caused by the binding effect of the mat but rather that the remains retained a natural coherence separate from the mat.

Table 2.1: Sequence of changes observed during the process of decomposition of fishes in nonmat control sediment and tanks with microbial mats. 0 = no changes observed; 1 = minor changes observed (i.e., some rays of the fin are broken); 2 = moderate decay; 3 = major decay observed (i.e., loss of color, loss of articulation).

Sample time (days)	Tank	Color	Head-body connection	Eye	Caudal fin	Dorsal fin	Scales
7	Non-mat	1	0	1	**1**	**1**	**0**
7	Mat	0	0	0	**0**	**0**	**0**
15	Non-mat	2	2	2	**2**	**2**	**1**
15	Mat	0	0	0	**0**	**0**	**0**
30	Non-mat	3	3	3	**3**	**2**	**2**
30	Mat	0	0	0	**0**	**0**	**0**
45	Non-mat	3	3	3	**3**	**3**	**2**
45	Mat	0	0	0	**1**	**1**	**0**
60	Non-mat	3	3	3	**3**	**3**	**2**
60	Mat	0	0	0	**1**	**1**	**0**
75	Non-mat	3	3	3	**3**	**3**	**2**
75	Mat	0	1	0	**1**	**1**	**0**
90	Non-mat	3	3	3	**3**	**3**	**3**
90	Mat	1	1	0	**1**	**1**	**0**

Based on qualitative data collected during observation (Table 2.1), hierarchical cluster analysis shows the percentage similarity of the sampled fish, classifying the corpses with a similar decomposition pattern into the same group. Figure 2.3 shows the strong difference between control and microbial mat fish. The control cluster contains fish from the control tank up to day 15. All the control fish, from day 30 to 90, are located in a subcluster denoting a similar, and advanced, stage of decay, which is further divided into two groups, depending on the decay time. Fish covered by microbial mats are grouped in a separate cluster. In this case, decay appears with a singular order, split into three groups: fish from (1) day 1 to 30; (2) days 45 and 60; and (3) days 75 and 90. Interestingly, the subcluster containing fish from days 1 to 30 was grouped with control fish #1, the only control sample that is not grouped in the decomposed cluster (see

Discussion). In fact, decay on control fish #1 was not noticeable. The three groups of microbial mat fish indicate progressive decay that follows a temporal sequence. The microbial mat fish did not attain a state of advanced decomposition comparable to that of the control fish within this experiment. Comparison between the control and the microbial mat fish was impossible after day 90 because of the advanced state of decomposition that characterized the remaining control fish.

The morphometric variables analyzed provide a good proxy for decay. Loss of thickness, for example, is a reliable variable because it may reflect decay collapse. ANOVAs of length, width, and thickness, using time and decomposition as the explanatory variables, show that the percentage loss of each variable is indicative of the influence of the mat on the decay rate (in all cases, $P < 0.001$) (Fig. 2.4). All morphological variables were influenced by time and decomposition setting (i.e., microbial mat versus sediment). Both microbial mat and control sediment tanks exhibited a similar development until day 15. The data show that significant decay occurred after day 15 (Fig. 2.4), and illustrate why the control sample #3 (day 15) is separated in the dendrogram from the subcluster containing control samples from day 30. On day 15, although the state of decay of the control fish was more advanced than that of the microbial mat fish, decay is not of the same magnitude as exhibited by subsequent control samples. This phase may be characterized by changes due to internal microorganisms (bloating phase) (Cambra-Moo et al., 2008). Although the bloating effect may have masked the loss of volume (length, width, and thickness), other qualitative observations reinforced a delay of 15 days before significant decay began. Subsequently, the differences in loss of volume increase, showing that decay in control samples in sediment becomes increasingly important through time, while decay is delayed in the microbial mat fish.

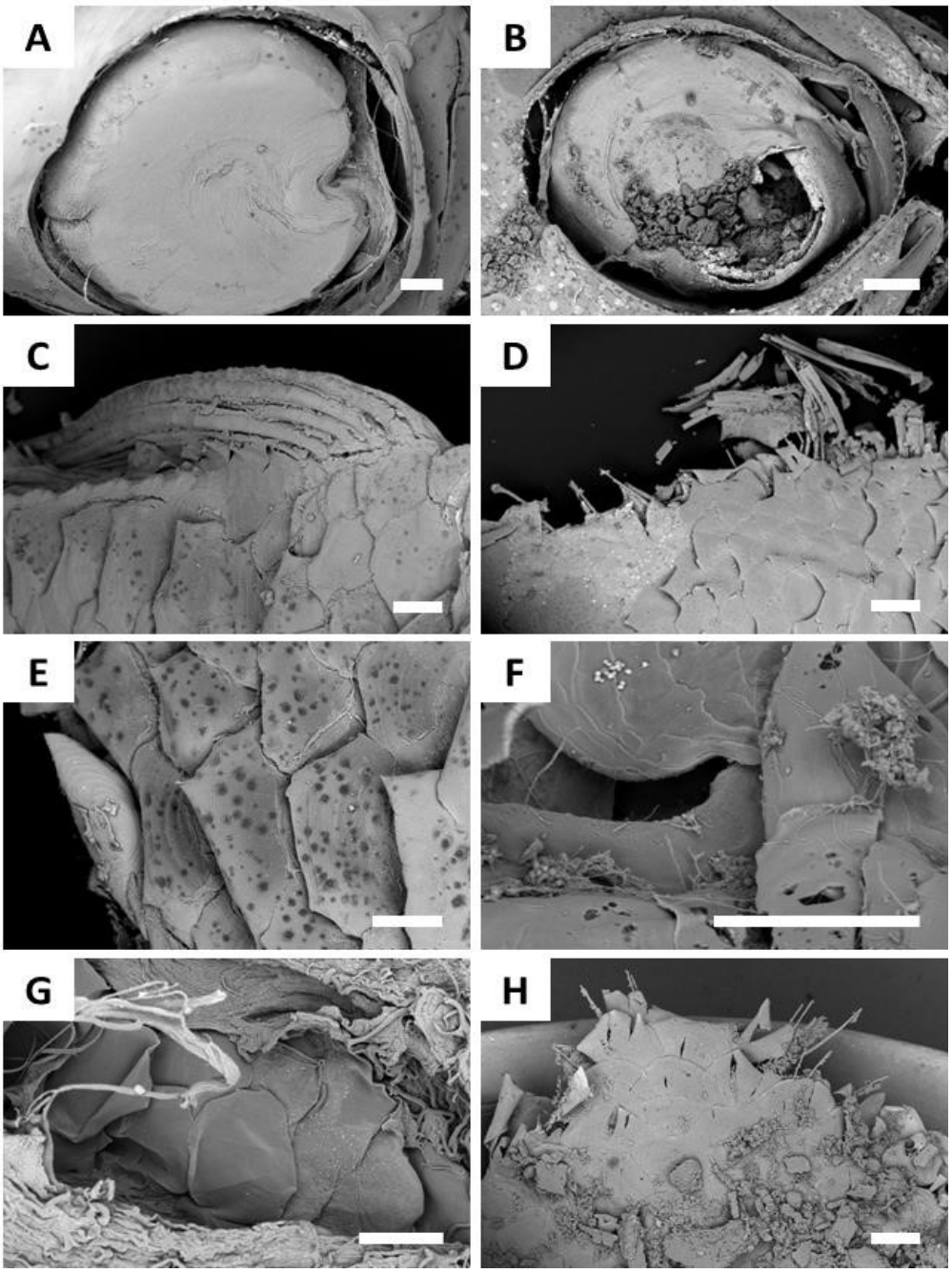

Fig. 2.2: SEM monitoring of the changes experienced by individuals during decomposition. Left- and right-hand columns correspond to samples on mat and on sediment, respectively. (**A–B**) detail of the fish's eye on day 7. The arrow marks the alteration of the eye in the control fish (arrow). (**C–D**) Detail of the dorsal fin (arrows) on day 7. (**E–F**) Detail of the fish's scales on day 30. F shows nonoverlapping and perforated scales (arrows). Dark blots in E are due to osmium tetroxyde precipitates. (**G**) Fish removed from the mat on day 90. Cut in the microbial coverage (a and b) enables observation of scales (arrow). The tegument is not broken or pierced and the integrity of the fish's body is maintained (no disarticulation observed), in spite of the damage caused by manipulation. (**H**) Detail of caudal fin on day 90. (Scale bar represents 250 µm.)

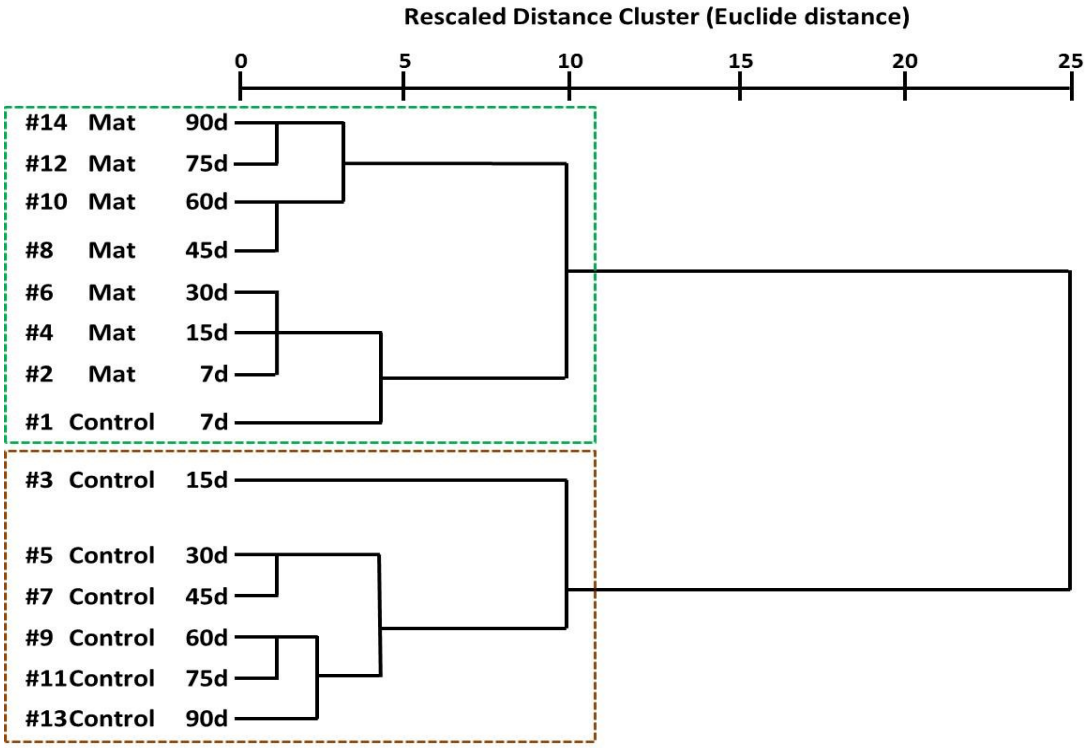

Fig. 2.3: Dendrogram obtained by hierarchical cluster analysis. The graph shows the differences in decay between the control fish and the microbial mat fish, which are separated in two clusters. Only control sample #1 (7 days) is placed in a cluster containing fish from the microbial mat samples, owing to the fact that initial differences in decay are not significant until after day 15.

Early Entombment and Initial Biostratinomic Processes

Two main structures are involved in covering the body of the microbial mat fish: a) a calcium-rich film, and b) the woven cyanobacterial layer that form a sarcophagus. The interrelationship of these two structures was difficult to observe because of the techniques employed for analyses (i.e., precipitation was monitored by SEM, while cyanobacterial entombment was examined with a stereomicroscope).

The importance of the natural formation of precipitates in mats has led to them to be regarded as essential to fossilization (i.e., bioinduced phosphatization, Briggs et al., 1993; Wilby et al., 1996; mineral replacement processes, Dunn et al., 1997). EDX showed that calcium carbonate was the main compound precipitated in mats (Fig. 2.5B), even though the Chiprana water is sulphate- and magnesium-rich. The precipitates of the control sediment are representative of the water composition ($MgSO_4$ and $MgCl_2$). Calcium precipitates were incidental and relatively poor. In contrast, Ca-rich precipitates appeared on day 7 on the carcass surface of the microbial mat fish. Small spherules of precipitates occurred in patchy regions of the surface of the fish (Fig. 2.5A). These spherules may have promoted the formation of a calcium coat that became a thin film by day 15 covering the whole carcass (Figs. 2.5C and D). The calcium enrichment of this coat was evident when broken areas of the film were analyzed (Figs. 2.5E and F). This calcium carbonate coat was initiated before the entire body became covered by cyanobacteria. This initial carbonate precipitation was likely induced by the

photosynthetic mat activity and the degradation of the extracellular polymeric substances (EPS) (Decho and Kawaguchi, 2003).

The growth of the microbial mat was inspected on days 7, 15, 30, 60, 90, 120, 270, and 540. Figure 2.6 shows entombment (i.e., the covering and entrapment of the carcass by cyanobacteria), and the progressive formation of a sarcophagus that reproduces the shape and size of the body. Cyanobacterial filaments start to weave a superficial green net on the body surface at day 15 (Fig. 2.6B), covering most of the body by day 30 (Fig. 2.6C). The observations made on days 270 (9 months) and 540 (18 months) illustrated that the fish had maintained integrity, with respect to tegument continuity, articulation and connectivity, and three-dimensional shape. The mat community that formed over the fish thickened over time. The fish was fully immersed in the mat at the interface of the green (oxic) and red (anoxic) layers (Fig. 2.6D) by day 270. At the time of the next observation (day 540, Fig. 2.6E) the fish was fully incorporated into the red anoxic layer.

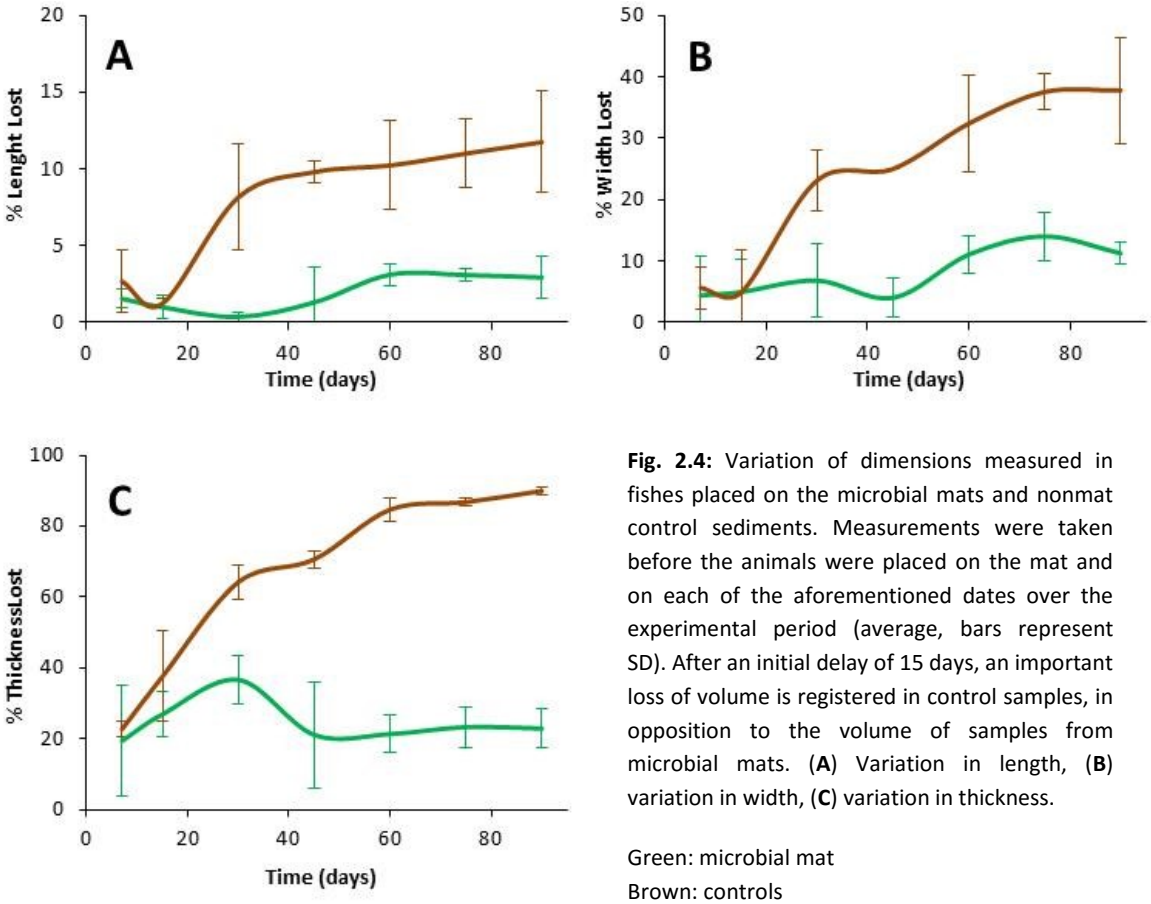

Fig. 2.4: Variation of dimensions measured in fishes placed on the microbial mats and nonmat control sediments. Measurements were taken before the animals were placed on the mat and on each of the aforementioned dates over the experimental period (average, bars represent SD). After an initial delay of 15 days, an important loss of volume is registered in control samples, in opposition to the volume of samples from microbial mats. (**A**) Variation in length, (**B**) variation in width, (**C**) variation in thickness.

Green: microbial mat
Brown: controls

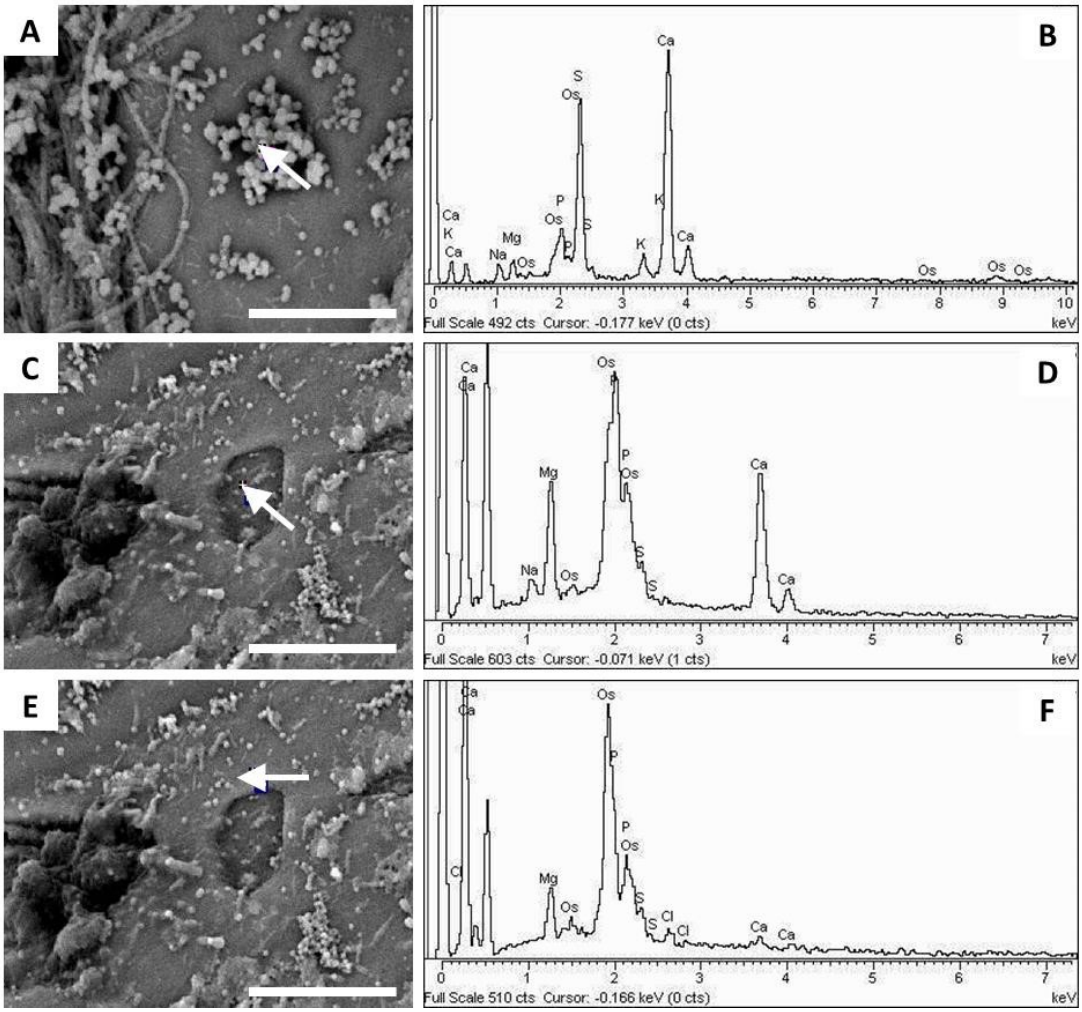

Fig. 2.5: SEM image showing precipitates over the surface of fish on microbial mat. (**A**) Spherules of precipitates (arrow a) on fish after 7 days. Arrow b shows some filamentous cyanobacteria. (**B**) EDX analysis of the precipitate of fish illustrated in (**A**). (**C**) Ca-enriched film on day 15 (arrow). (**D**) EDX analysis of the precipitate of fish illustrated in (**C**). (**E**) Hole in the film (arrow) showing a surface poor in Ca after 15 days. (**F**) EDX analysis of the precipitate of fish illustrated in (**E**). Presence of osmium tetroxide and phosphate in samples is due to fixation. Signal numbers correspond to the area analyzed. White arrow shows the point analyzed by the EDX. Scale bars represents 20 µm

Long-Term Monitoring

To determine the extent to which the integrity is maintained throughout the initial biostratinomic stages, MRI samples (day 270 and day 810) were compared with a recently dead individual (Fig. 2.7A). MRI of mat fish reflected its external integrity, and strikingly, the fish preserved its internal skeletal organization and soft organs. In an MRI of the control fish, only medullar and eye fragments were recorded. The control fish later provided an obscured, weakly contrasted image, contrary to the perfectly contrasted microbial mat fish (Fig. 2.7D). The contrast reflects the hydration level of the inner organs (e.g., sclerocorneal capsule, swim bladder, and muscle somites) indicating that decomposition is much slower under these circumstances (Figs. 2.7B and E). Nevertheless, the contrast faded for the 810-day microbial mat specimen (Fig. 2.7C). At

that stage, the microbial growth over the body was probably already compressing it, thereby explaining its loss of volume and its dehydration.

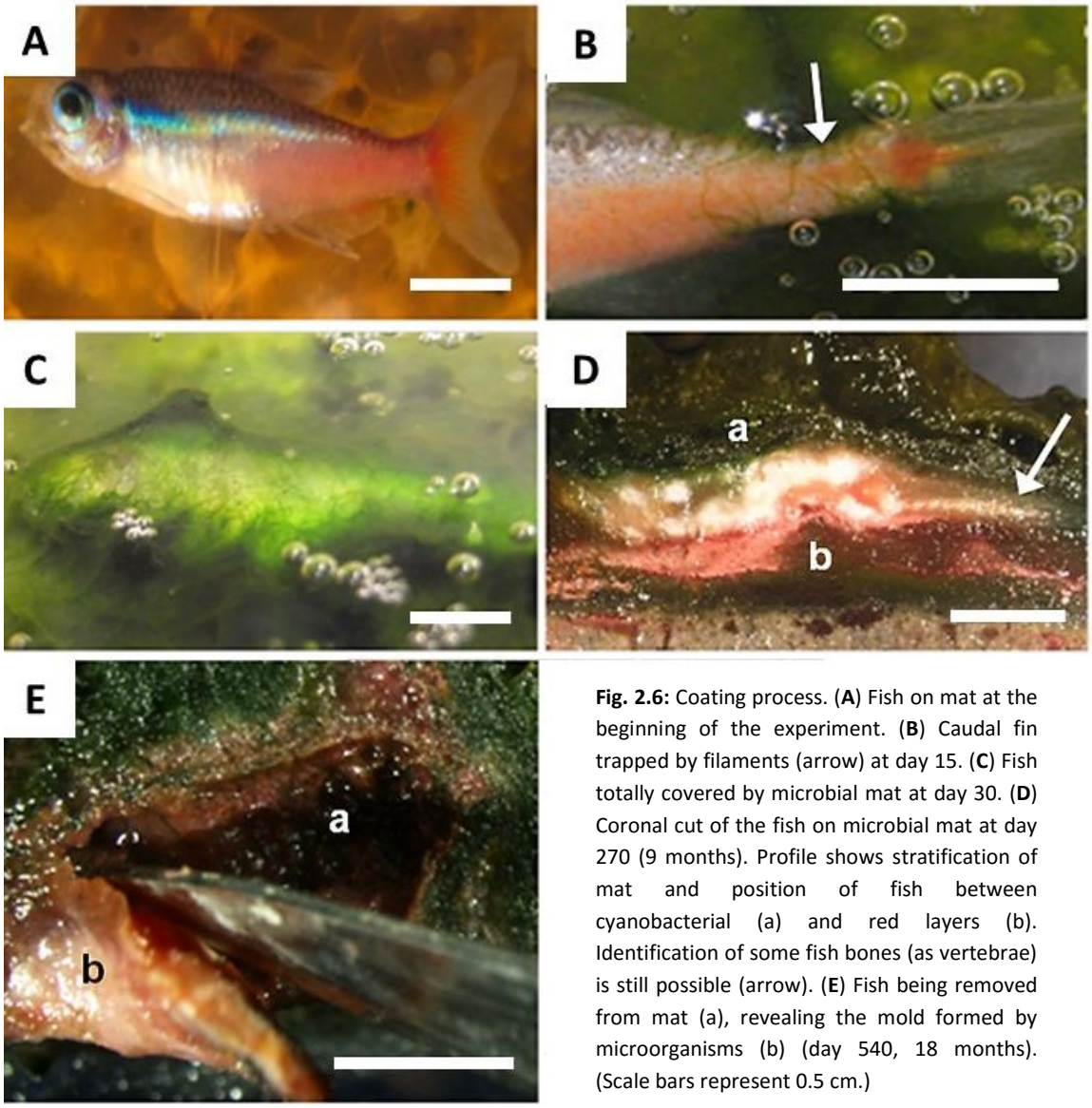

Fig. 2.6: Coating process. (**A**) Fish on mat at the beginning of the experiment. (**B**) Caudal fin trapped by filaments (arrow) at day 15. (**C**) Fish totally covered by microbial mat at day 30. (**D**) Coronal cut of the fish on microbial mat at day 270 (9 months). Profile shows stratification of mat and position of fish between cyanobacterial (a) and red layers (b). Identification of some fish bones (as vertebrae) is still possible (arrow). (**E**) Fish being removed from mat (a), revealing the mold formed by microorganisms (b) (day 540, 18 months). (Scale bars represent 0.5 cm.)

DISCUSSION

Experimental Outcome

Microbial mats need to be grown for a long period of time for their complex structures to fully develop. Few experiments have been carried out specifically with vertebrates in continental microbial mats. Most of the taphonomic analyses of fish have involved marine environments (Viohl, 1994; Bieñkowska, 2004; Esperante et al., 2008), although there are a few exceptions (Mancuso, 2003; Martín-Abad and Poyato-Ariza, 2010). In addition, these previous investigations used single-layered biofilms. Therefore, it is somewhat difficult to establish a comprehensive referential framework to discuss comparatively and in detail the resulting biostratinomic changes.

Fish fossils commonly occur within the habitat wherein their carcasses are found, and hence are important for inferring environmental conditions (Wilson, 1988). Thus, the analysis of carcasses in sediment covered with water and on microbial mats introduces new elements to the study of fish taphonomy in exceptional deposits. Our experiment helps to establish a firm basis for the framework needed for collecting data (i.e., sample size necessary to enable changes in sediment fish to be compared, and also more natural experimental conditions). Long-term observations in particular are based on only a few analyzed samples. Within our experiment, MRI analyses were instrumental in determining the coherence of fish carcasses entombed in microbial mats for periods up to and exceeding two years.

It has been postulated that the presence and activity of microbial mats plays a key role in the optimal conservation of remains. Factors inferred rather than tested in microbial mats (i.e., protection, delayed decay, and mineralization) have been presented as necessary conditions for preservation (Gall, 1990; Wilby et al., 1996; Gehling, 1999; Briggs, 2003a, Briggs et al., 2005; Martill et al., 2008). However, previously there was neither direct information nor experimental description of the temporal sequence of the processes in which microbial mats develop and contribute to carcass preservation.

Even if a fish corpse floated for an interval prior to settling on a benthic mat, 15 days elapsed before notable decomposition occurred (Fig. 2.3). Obviously, the time interval depends, in part, on the water composition. For instance, it has been inferred that saline water should delay bacterial growth (Martill et al., 2008; Martín-Abad and Poyato-Ariza, 2010). Once the fish is on the surface of the mat, EPS and filamentous cyanobacteria contribute to attach the corpse to the mat. This process is different from transport and trapping of the body to the sediment by single-layered biofilm, as has been described elsewhere (Martínez-Delclòs et al., 2004). This simple biofilm grows over carcasses and sinks the body, but it is not implicated in fixation to the bottom. At this phase, gas production may help to refloat corpses. Therefore, quick fixation and the coating process (from day 15) appear to be two major factors preventing disarticulation. Rapid burial by sediment may significantly prevent decay as well (Seilacher et al., 1985). Because the control fish were kept subaerially during this experiment, the influence of rapid burial by sediment was not contrasted.

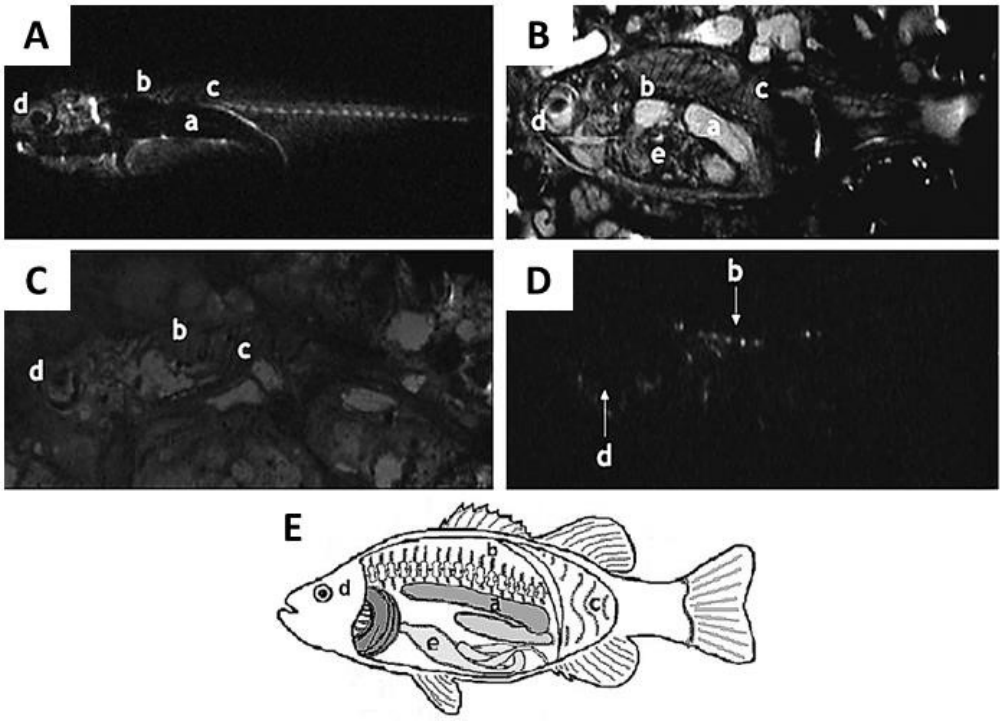

Fig. 2.7: Magnetic resonance imaging of fish. Brightness of the observation reflects the degree of tissue hydration. In this T2-weighted scan, the water is shown as a light tone and the fat tissue in grey. (**A**) Fish at the beginning of the investigation. (**B**) Fish in microbial mat after 270 days (9 months). (**C**) Fish trapped by mat after 810 days (27 months). (**D**) Decomposed fish on control, sediment. Only some sections of vertebrae and eye socket are visible (arrows). Scale bars for A–D are 1 cm. (**E**) Sketch of the inner organization of the fish, for comparison with MRI observation. In this image: a = swim bladder; b = spinal cord; c = muscle; d = eye sclerocorneal capsule; e = intestines.

Ca-rich precipitates on the body surface occur as soon as coverage begins. These results are consistent with previous sedimentological and ecological experiments that demonstrate the involvement of microbial activity in $CaCO_3$ precipitation, even in the absence of carcasses (Kühl et al., 2003; Dupraz and Visscher, 2005; Dupraz et al., 2009). The observed precipitated particles (Fig. 5A) are organized in a similar way to those $CaCO_3$ spherules formed by induced bioprecipitation (Krumbein et al., 1977; Decho and Kawaguchi, 2003). EPS produced by microbes seem to be closely related to this process. During microbial production and secretion of these compounds, Ca and other ions are sequestered. When EPS become degraded, these minerals are released into the environment (water). This sudden increase in the concentration of these elements would exceed the maximum (saturation), making precipitation easier (Kühl et al., 2003). O'Brien et al. (2002) hypothesized, from observations of exceptional insects and fossil plants, that EPS would probably be decisive in preservation. This experiment provides evidence that EPS plays an important role because carbonate precipitates precede the cyanobacterial net. The formation of these spherules may be related to the construction of an integral carbonate film. This kind of film maintains the structure of the corpses, forming a microenvironment that surrounds them, reducing the decay rate and

promoting mineralization and the formation of molds. The formation of such an extended calcium film covering rather large bodies would not be possible in single-layered biofilms. Previous observations by Peñalver (2002) demonstrated that the microbial photosynthetic single-layered biofilms formed over floating carcasses do not resist turbulence and thus lose their integrity. This difference between microbial mats and single-layered biofilms is one of the most important factors in the difference between these structures in carcass preservation.

The green microbial mat cover may eventually become a sealed sarcophagus. In such a situation the degree of protection is significant. In the natural environment, this protection would make scavenger attack or erosion and transport by natural currents difficult. Thus envelopment by microbial mats is a key factor preventing disarticulation, as suggested by O'Brien et al. (2008). The protection would also be influenced by the degree of hydration which is maintained by the mat embedded in water, and probably boosted by the presence of EPS. Hydration appears also to be important to corpse integrity (discussed below).

Anoxia is another important factor for exceptional preservation. It minimizes disarticulation by reducing the decay rate and by preventing the activities of scavengers (Fregenal-Martínez and Buscalioni, 2009). Microbial mats can create anoxic conditions in three ways: (1) Because of their stratified structure, there is a compartment rich in reduced compounds beneath the green oxic layer. The decomposition rate is reduced in this compartment because anaerobic metabolism is less energetically efficient. In mats, the oxygen decay occurs at a depth of 1–3 mm. (even during light intervals) while in nonmat sediment the lack of oxygen occurs at a depth of 6 m. (2) Because the anoxic microbial layer prevents contact with the oxygenated water column or even the oxygen produced by the mat itself, the sediment under mats is in constant anoxic conditions. Under some conditions (for example, at night when photosynthesis enters a stand-by phase), reduced elements (such as H2S) from the sediment or the anoxic layer could make the entire water column (or at least its deepest zone) anoxic. (3) Due to the formation of a Ca-rich film, the carcass would be isolated and protected from oxygen produced by cyanobacteria.

Figure 2.8 summarizes the processes related to entombment and replication (corpse mold) of a fish carcass that promotes and enhances exceptional preservation. A biological coating of EPS and filamentous cyanobacteria quickly covers carcasses. After entombment, the microbial coverage preserves the shape and integrity of the organic material, providing protection against abiotic agents (wind, water flow). Biological degradation is also possible, removing the fish from the surface and hiding the corpse. This protection is amplified by the anoxic conditions that are inherent to microbial mat organization. In addition, anoxia affects the delay in the decomposition rate and induces carcass replication promoting bioprecipitation of Ca compounds. This slowdown in the rate of decomposition spans the time for replacement and mineralization of the remains favoring preservation.

Implications for Fossil Preservation

The principal taphonomic implication of the role of microbial mats is preservation in the first stages of fossilization and body integrity. These initial stages, subject of this paper, are essential in fossilization, regardless of the deposit properties (fossil composition, organic origin, mineralization, chemical and physical conditions). We apply the term integrity to those features (tegument continuity, connectivity, and three-dimensional shape) that are necessary for exceptional fossilization. While carcasses on nonmat sediment lose their consistency, becoming unstructured after day 30, bodies in microbial mat preserve their integrity and remain coherent. Morphometric analysisrevealed that fish on microbial mats maintained, to a large extent, their original size and volume (length, width, and thickness) over the experimental period (3 months for quantitative analysis and 27 months for observational description) and this differed notably from the control. Integrity may also be associated with the nature of the water. Salinity (particularly hypersalinity) has been suggested as a factor for fish death and exceptional preservation (Bieñkowska, 2004; Martill et al., 2008). Results of experiments comparing fish decomposition in marine and fresh water tanks show that body integrity is apparently higher in marine water, but carcass turns to a gel when perturbed (Martín-Abad and Poyato-Ariza, 2010). Thus, water alone cannot be considered a contributing preservation factor; other external conditions related to burial must also be met. Integrity is also favored by formation of a microbial mat sarcophagus and encasement by a calcium-rich film. These two factors enable the maintenance of the three-dimensional organization (preventing flattening), hydration, and anoxia. MRI images show that after 270 and 810 days the integrity of external and internal organs did not significantly change. The tegument was not pierced and the fish remained fully articulated. Furthermore, a clear outline of the fish's inner organs, such as the sclerocorneal capsule of the eye, the swim bladder, and muscle somites, were still present and visible due to hydration. Although at day 810 the fish was less hydrated (the MRI image was not sharply contrasted; Fig. 2.7C) and the body was more flattened (35% of thickness lost), this flattening was gradual and occurred before compression induced by burial. These gradual changes (slow decay) are important for keeping the body in the best preservation condition (Briggs et al., 1997).

In summary, the role of microbial mats at the initial stage of fossilization is related to a set of favorable conditions of preservation, namely the absence of physical distortion, necrokinesis (lateral transport), disarticulation, or dispersion of elements. These favorable conditions are facilitated because microbial mats are involved in distinct biostratinomical processes: (1) biostratinomic encrustation, which involves the envelopment by cyanobacterial filaments; (2) precipitation of calcium, which is promoted by EPS and leads to the formation of a calcareous concretion covering the carcass; (3) concretional mold formation (replicating the external shape of the fish). In fact, whereas concretional molds were not directly observed in our experiment, they have been noted elsewhere in studies conducted on shrimps under controlled laboratory conditions by Briggs and Kear (1993). Thus, the physicochemical properties of

microbial mats ensure that these early preservational processes will take place regardless the environmental conditions of the deposits.

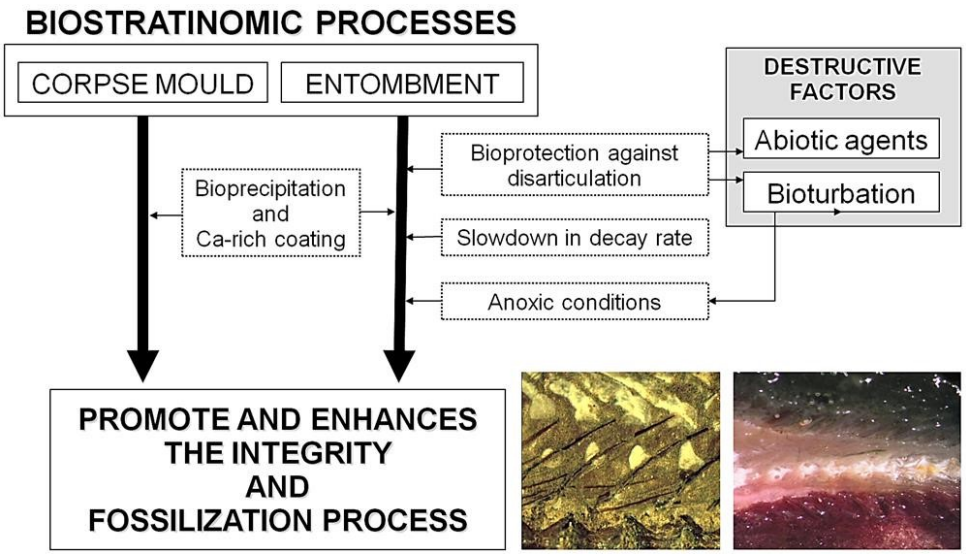

Fig. 2.8: Diagram summarizing the role of microbial mats in exceptional preservation. (**A**) Detail of caudal fin of a fish covered by microbial mat after 9 months. (**B**) Fossil found at Las Hoyas (Spain) showing somite organization.

The experiments discussed herein provide a detailed description of the initial stages of fossilization under the influence of microbial mats. Differences in decay between fish specimens on microbial mats and in the control group become apparent after day 15. Fish on microbial mats exhibit a delayed decomposition, maintaining external body integrity. MRI permits the recognition of the exceptional preservation of the internal organization and soft organs in microbial mat fish after 27 months, whereas control fish were rendered down to a few disarticulated bones. The sequence of processes related to entombment and replication started with carcasses covered quickly by EPS and filamentous cyanobacteria. Microbial coverage provides protection against biotic and abiotic agents, boosted by the anoxic conditions inherent in settings where microbial mats commonly develop. Anoxic conditions delay decomposition and induce precipitation, promoting carcass replication. As a consequence, the interaction of all these factors (protection, anoxia, delay in decay, and bioprecipitation) are essential to promote and enhance integrity, and exceptional preservation, a common factor in all Konservat-Lagerstätten.

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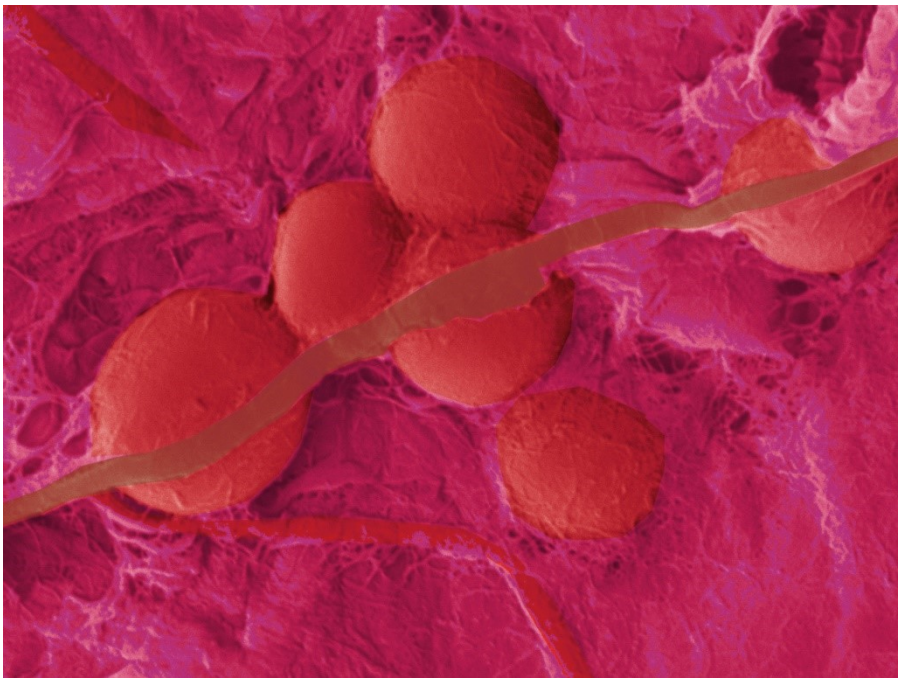
CAPÍTULO 3

THE EFFECT OF MICROBIAL MATS IN THE DECAY OF ANURAN: TRYING TO UNDERSTAND THE FOSSIL RECORD



Artículo en preparación:

Iniesto M, Villalba I, Guerrero MªC, Buscalioni AD, López-Archilla AI. The effect of microbial mats in anuran decay: Trying to understand exceptional preservation

ABSTRACT

The present study addresses the implication of microbial mats in early fossilization through taphonomic experiments with frogs. Comparison of several morphometric variables has shown statistical differences in the degree of decay between frogs over mats and over sediments. At day 240, control animals were just a dark organic sheet over the sediment surface, while in mat remains were preserved by a microbial cover after 1080 days. Inside the microbial sarcophagus, frog carcasses remain articulated and show soft tissues such as skin, tendons, muscle and even bone marrow (fatty and mesenchymal tissues) preserved up to three years. In addition, several organs such as the tectum of the brain have been mineralized into a Ca-rich carbonate phase. Remarkably, the same kind of bioprecipitates can be found in brains of fossil frogs from Libros (Miocene, Spain) and Las Hoyas (Lower Cretaceous, Spain). Based on our results, we discuss whether the presence of these microbial communities is key in organic preservation and/or authigenic mineralization of biological remains. Our outcomes help explaining the exceptional preservation of several specimens of the fossil record coming from systems supporting mats.

Keywords: delay in decay, excepcional preservation, fossilization, organic preservation, mineralization

RESUMEN

El presente estudio analiza la implicación de los tapetes microbianos en la fosilización temprana a través de experimentos tafonómicos con ranas. La comparación de distintas variables morfométricas ha mostrado diferencias estadísticas en el grado de descomposición entre las ranas colocadas sobre tapetes microbianos y sobre sedimentos sin tapete. Los animales control se convirtieron en una simple y fina lámina orgánica sobre la superficie del sedimento a los 240 días, mientras que los restos sobre tapetes se preservaron después de 1080 días gracias a la formación de una cubierta microbiana. En el interior de este sarcófago microbiano, las ranas permanecieron articuladas y mostraron algunos tejidos blandos tales como la piel, los tendones, los músculos e incluso la médula ósea (tejidos grasos y mesenquimales) conservados después de tres años. Además, una parte del cerebro, el tectum, fue mineralizado en una fase rica en carbonato de Ca. Sorprendentemente, el mismo tipo de bioprecipitados se puede encontrar en el cerebro de fósiles de ranas procedentes de Libros (Mioceno, España) y de Las Hoyas (Cretácico Inferior, España). En base a estos resultados se discute si la presencia de los tapetes microbianos es clave en la preservación y/o la mineralización autigénica de restos biológicos. Nuestros resultados sirven para explicar la preservación excepcional de algunos especímenes del registro fósil procedentes de sistemas donde se desarrollaron tapetes microbianos.

Palabras clave: fosilización, mineralización, preservación excepcional, preservación orgánica, retraso en la descomposición

INTRODUCTION

Taphonomic studies are essential to properly interpret the fossil record. Most of the research is carried out by means of the direct observation of fossil specimens but the number of articles based in experimental taphonomy is rising. However, only a few of them have actually been conducted using vertebrates as case study, in particular adults, although they appear as common fossils in many Konservat-Lagerstätten (Archer et al. 1989, Buscalioni et al. 2008, Martill & Bechly 2007). Some of these experimental studies supported the idea that microbial activity played a capital role in the taphonomic process; for instance, in the preservation of soft tissues as a result of authigenic mineralization (Briggs & Kear 1993, Hof & Briggs 1997). The microorganisms involved in the assays were taken from sea or lake sediments (e.g. (Briggs & Kear 1993, 1994; Hof & Briggs 1997, Martin et al. 2003, 2005; Wilby et al. 1996), and/or just the inner microbiota from the decaying bodies of the experiment (Butler et al. 2015) and grew forming simple biofilms in most of these assays. However, these simple biofilms are labile and lack most of the properties of more complex ones, such as microbial mats. Microbial mats are highly diverse stratified communities, mainly composed by primary producers (photoautotrophic and/or chemoautotrophic) and consumers/decomposers (heterotrophic). The existence of a food web that carried out an energy flow and a closed nutrients cycle let to consider these communities as an ecosystem on their own (Stolz 2000). Most of the physicochemical properties of phototrophic microbial mats, are conditioned by the vertical gradient generated by light penetration as well as the metabolic activity of the microorganisms (Cohen & Rosenberg 1989, Wierzchos et al. 2006). According to these inherent properties of mats, some authors stated that these communities could play a key role in fossilization, for example, in obrution (i.e., fast growth that enables rapid coverage of carcasses) (Seilacher et al. 1985) and also in authigenic mineralization for their ability to lithify (e.g. Chafetz & Buczynski 1992; Decho & Kawaguchi 2003; Dupraz & Visscher 2005; Dupraz et al. 2009).

Although the regular fossil record is biased and is dominated by shelly organisms, Konservat-Lagerstätten present many rests belonging to vertebrates. Several remains are slightly disarticulated, such as skeletons of mammals, reptiles or birds, occurring for instance in Rubielos de Mora (Miocene, Spain) (Mcnamara et al. 2012); however, most fossils found in Konservat deposits are fully articulated and preserved soft tissue. The earliest vertebrate fossils come from the Amadeus Basin (Lower Ordovician, Australia [Janvier 2003]). They are mostly microvertebrates, mainly agnathan and chondrichthyans, two different groups of fish (Young 1997). In fact, remains of fish are frequent and widespread in the fossil record of Konservat Laggerstätten, for example in Tlayúa Quarry (Cretaceous, México) [Alvarado-Ortega et al. 2007]), Riversleigh (Cenozoic, Australia [Archer et al. 1989]), Las Hoyas (Lower Cretaceous, Spain [Gupta et al. 2008; Buscalioni & Fregenal-Martínez 2010]), Soom Shale (Upper Ordovician, South Africa [Alridge & Theron 1993]), Green River Formation (Eocene, USA [Ferber & Wells 1995]) or the Crato Formation (Lower Cretaceous, Brasil) [Brito 2007]). Although rests belonging to other groups of vertebrates are also prevalent, those belonging to anuran

are scarce in the geological record, normally restricted to fossilized bones often broken and in fragmentary conditions (Báez 2013). This paucity hinders unravelling the history of frogs. However, there are several deposits where fossils of amphibians are preserved and fully articulated, such as Las Hoyas (Báez 2013) o Río Pichileufú (Eocene, Argentina [Gómez et al. 2011]). In the exceptional case of the Spanish site of Libros (Miocene, Spain) some adults and tadpoles of anurans specimens are preserved as complete rests, with soft tissue, and a carbonate outline that shapes the original carcass (McNamara et al. 2009, Mcnamara et al. 2010). In this specific deposit, the preservation of some anurans is so exceptional that even the bone marrow is maintained (McNamara et al. 2006).

Taphonomic experiences are essential to understand fossil bias and the potential for preservation of different tissues and/or groups of organisms. For example, some experiments conducted with arthropods have shown that soft tissues can be selectively mineralized (Briggs & Kear 1993, Hof & Briggs 1997) and these outcomes would also be valid for vertebrates decay. In order to understand the decay sequence, experiments analysing the evolution of carcasses are useful to fully unveil how exceptional preservation is possible. In the case of vertebrates, experimental taphonomic approaches are scarce and frequently conducted with fishes (Breder Jr. 1957, Martín-Abad 2015); only a few of them are focused on other groups (e.g. birds, in Cambra-Moo & Buscalioni 2003). In addition to the lack of knowledge on vertebrates decay, the relationship between microbial mats and preservation is usually neglected, though several fossils are embedded in an organic veil which has been explained as the matrix of a mat (McNamara et al. 2009). Several cells of the mats have been reported laying over fossil specimens as in the case of Pelecanimimus in Las Hoyas (Briggs et al. 1997). Recent experiments have supported the idea that inherent properties of microbial mats promote the exceptional preservation of tissues. These outcomes have focused in the description of: 1) the growth of mat over animal carcasses and the formation of a sarcophagus (Iniesto et al. 2013); 2) the formation of impressions of body surface in the framework of mat; 3) the replication of some body parts during microbial colonization and; 4) the authigenic formation of a poorly crystalline Mg-silicate phase in relation to the decay of carcasses (Iniesto et al. 2015b), but these experiments were conducted with fish (Iniesto et al. 2013, 2015a,b). Although the model explaining the implication of mats in taphonomy has, in consequence, a robust experimental support, it has to be implemented in more experiments in order to check the possible variations due to animal size or animal group, environmental conditions, etc. The present paper tries to unveil the pattern and sequence of decomposition of anurans in microbial mats, aiming to decipher their potential for exceptional preservation. It also provides a comparison of the conservation process in fish and frogs, in order to explain the major differences observed in the fossil record in terms of preservation. The final goal is to help in the interpretation of the fossil record by contrasting our experimental results with similar fossilized remains.

MATERIAL AND METHODS

In order to achieve the objectives depicted above, several bodies of frogs were placed on microbial mats previously grown in laboratory, or over sediment without mats as controls.

Microbial mats origin and growth in laboratory

Samples of microbial mat came from "La Salada de Chiprana" (Zaragoza, Spain). This shallow lake is a permanent system of endorheic origin in a semiarid region of the Ebro river depression (Aragon, NE Spain), and its waters are hypersaline (30–70 ‰). The bottom of the lake is colonized by microbial mats from the shoreline to a water column depth of 1.5 m (Guerrero et al. 1991), favoured by the extreme physical and chemical conditions. Additional details concerning physicochemical and biological characteristics of this site can be found in Vidondo et al. (1993), Jonkers et al. (2003) and Ludwig et al. (2005).

Microbial mats were grown in laboratory under light and temperature controlled conditions. Tanks measuring 40 x 20 x 20 cm were filled with a 2 cm layer of sediment from the lake over a base of limestone of 2–3 cm. Four tanks were used, three with microbial mats and one without mat used as control. Soon after collection, microbial communities' samples were ground up using a basic mixer (T25 IKA Labortechnik) and the resulting mixture placed over the corresponding sediment in three of the tanks. Tanks were finally filled with water from Chiprana until the column had a depth of around one centimetre. pH, dissolved oxygen (DO), conductivity and temperature were monitored using specific probes (WTW pH 3110 SET 2, Oxi 315i / SET, 2C10-0011 and Cond 330i respectively; temperature was measured with the oximeter) during all the experiment. Sterilized deionized water was added to the tanks during the course of the experiment to avoid strong variations in water level and salinity as a result of evaporation. Conductivity and temperature were relatively constant during experimental times (mean 50.1 ± 2.3 mS·cm-1 and 23 ± 0.5ºC respectively). Tanks were illuminated by a 50W halogen lamp (Osram Decostar 46870WFL) except for the tank used as control, kept in darkness to prevent the growth of phototrophic microbial mats from forms of resistance present in the sediment. Illumination intensity of surfaces was approximately 300 mmols·m-2·s-1 (±1 mmols·m-2·s-1). Tanks were exposed to a 10/14 h light-dark cycle. Microbial communities were allowed to grow until stabilization, determined by the exhibition of a structure and composition similar to original mats.

Experimental taphonomy

The experiment was carried out using the anuran *Hymenochirus boettgeri* (African dwarf frog) as a model, according to its morphological characteristics; this facilitated the

assessment during the decay process. Frogs were killed following standard animal care protocol used at Universidad Autónoma de Madrid. Dead bodies (9 in each tank, Ntotal=36) were laid on the surface of microbial mats (see below) with a 2.5 cm gap between specimens. Individuals were randomly chosen and analysed at days 7, 15, 30, 120, 242, 540 and 1080 of the experiment (one per tank per time). Two frogs remain currently into the sarcophagus in each tank with mat, wainting to be extracted in the future to check their integrity and/or damage. There are no frog remains in the control tank. To compare the decay process of frogs between tanks with and without mats as well as with the results of previous experiments with fish (Iniesto et al. 2013), several quantitative and qualitative observations of the specimens were made. Quantitative data were obtained from the following morphometric variables: total length, width at the base of the head, width of the pelvis, femur length, tibia length, and head, abdomen and thigh thickness. Measurements were taken with a digital caliper. Complementary description of the biostratinomic processes associated with entombment in mats was also performed. When frogs were covered by the mat, the carcasses were separated from the mat by cutting a thin layer of the sarcophagus under the stereo-microscope. In controls, frogs were removed with a small spatula, in order to minimize damages, and cleaned with drops of water.

Qualitative observations were based on changes showed by the decaying frogs according to handling and modifications in several features. These parameters were determined on the basis of: 1) tetrapod disarticulation sequences definited previously by Cambra Moo & Buscaloni (2003) and Cambra Moo et al. (2008) and; 2) variables introduced after preliminary test carried out with frogs in order to implement the study to anuran decay. These morphometric parameters were: swelling; preservation of initial pigmentation; new pigmentation; visualization of the linea alba (i.e., a fibrous structure that crosses the midline of the abdomen in vertebrates, mostly made by collagen connective tissue [Axer et al. 2001]); presence of large amounts of exopolymeric substances (EPS); wrinkles in skin and grade of articulation. The pull of data retrieved allowed checking the influence of microbial mats in frog decay. An ANOVA test performed with quantitative data to compare the loss in volume (determined by morphometric measurements) between controls and frogs decaying in mats all over the time of experimentation. The null hypothesis tested, i.e. “decay in microbial mats did not show differences with control decomposition”, was rejected with a p-value ≤0.01. As the factor “time” had more than 2 groups, a comparison by pairs was carried out with a Bonferroni test. Results of the qualitative monitoring were analysed with a Hierarchical Cluster Analysis to check resemblances in the variables between samples. The resulting Dendrogram allowed the description of the sequence of changes during decay according to the presence or absence of mats. The whole statistical analysis was performed using the analytical software IBM SPSS Statistics 22.

Scanning electron microscopy (SEM) and Energy dispersive x-ray spectrometry (EDXS)

Electron microscopy was used in order to track both occurrence of precipitates in the sarcophagus and carcasses integrity. For these analyses, samples of frogs were observed within microbial sarcophagi. The whole block was fixed in glutaraldehyde (2%) over 48h and dehydrated in a gradual series of ethanol baths (50, 70, and 3x100%). Prior to dehydration, samples were sectioned along a coronal plane. Several parts such as legs were embedded in epoxy resin, sectioned with a diamond saw blade and polished with diamonds down to ¾µm in size. All the samples were then coated with gold. Images and analyses were collected in backscattered and secondary electron modes using a Hitachi S3000N operating at 15 kV, with a 60 µm aperture at a working distance of ~15 mm. Elemental compositions were determined using energy dispersive x-ray spectrometry (EDXS) with an Oxford Instruments INCAx-sight system.

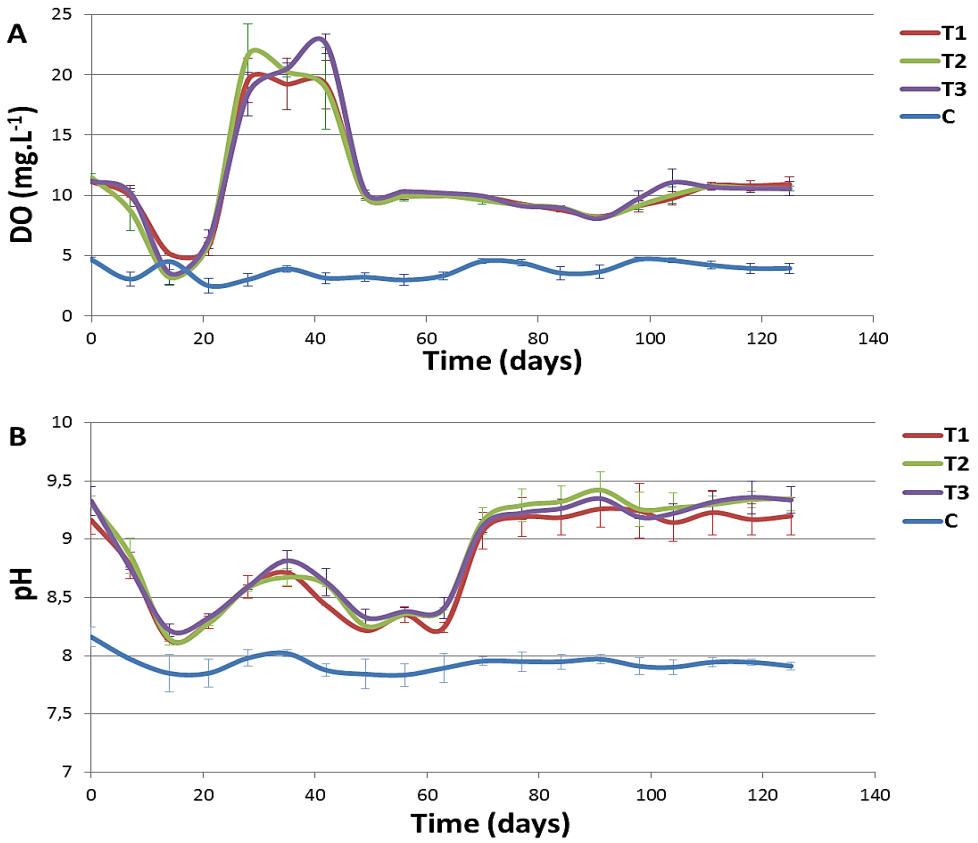


Fig. 3.1: Variation of DO (**A**) and pH (**B**) in the course of the experiment in control and microbial mat tank water column. While pH and DO are stable in controls, tanks with mats showed great variations through time. The placement of carcasses is followed by a decrease in both pH and DO until the day 14, when measurements recorded a high rise that stand for two weeks. After that, values returned to baseline.

RESULTS

Changes in the water column and growth stimulation of the mat

The placement of frog carcasses over the microbial community led to local stimulation of the mat, especially of the oxigenic photosynthetic microbial populations. This rise in the development was in the basis of the early green-darkening of the upper layer during the first week (Supplementary Fig. S3.1). The increased mat activity produced environmental changes in the water column. DO and pH measured in the course of the experiment were very similar in the three mat tanks but differed from the control tank. In control tanks DO remained stable and low (below 5 $mg.L^{-1}$), but in mat tanks it varies much more. An initial decrease until day 14 (Fig. 3.1A) was followed by an increase up to 15 $mg.L^{-1}$ between days 14 to 42. After day 42, the DO went down again to baseline values (around 10 $mg.L^{-1}$), and these values continued relatively constant during the following weeks. Similar variations were observed in pH (Fig. 3.1B): later carcasses were laid in the mat surface, the water column acidified from 9.3 down to 8.1 at day 14. Subsequently, pH increased slightly (to 8.7) at day 35 and increased again after day 63, reaching the initial values. After this last rise pH remained almost constant until the end of the measurements. Regarding the controls, pH was more stable and lower (near 8) than in mats tanks.

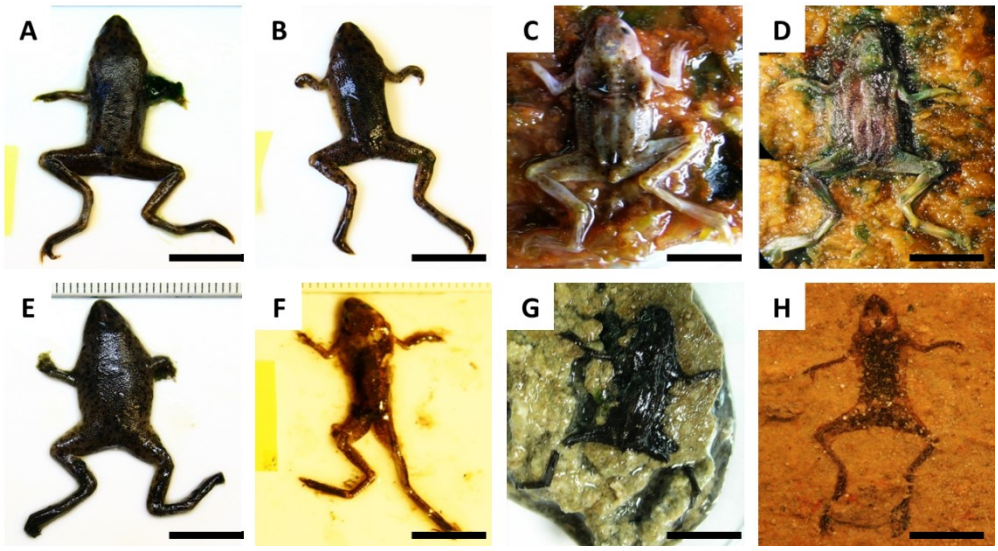


Fig. 3.2: Summarize of the evolution of carcasses in the course of the experiment. (**A**) and (**E**) Carcasses after day 15 over mat and sediment respectively. (**B**) and (**F**) decay state of frogs after 30 days in mat and sediment respectively. Control frogs were quite damaged and broke easily. (**C**) Frog removed from the microbial envelop at day 120, completely articulated and preserving soft tissues such as skin while control (**G**) is completely decayed. (**D**) Frog after its removal from the sarcophagus after 240 days. It is noticeable that the carcass is still articulated as at day 120. (**H**) Control frog at day 240, showing a massive decay, becoming a dark shadow over the sediment surface. Scale bars represent 1 cm.

Soon after the placement of anuran in the system (25-30 days), carcasses were trapped by bacterial filaments from the upper cyanobacterial layer (Supplementary Fig.

S3.2B and C), forming a microbial sarcophagus (Supplementary Fig. S3.2D-F). Controls showed a darkening of the sediments around the animals (Supplementary Fig. S3.1C), consequence of increasing the decay and the production of sulphide which reacted with the Fe contained in the sediment.

Taphonomic changes

The comparison of integrity and general state of corpses over sediment (controls) and mats in the course of the experiment can be seen in Fig. 3.2. Frogs on mats at day 15 are fully articulated and its integrity did not present changes (Fig. 3.2A), whereas in controls, although remained articulated, were swelled due to decay (Fig. 3.2E). These differences increased until day 30 when animals in controls broke during manipulation, and the skin was pierced (Fig. 3.2F). In contrast, frogs covered by mat kept the integrity, allowing complete handling during data collection (Fig. 3.2B). Frogs from mat showed an evident flattening at day 120, but maintained the original consistency (Fig. 3.2C), whereas controls were so decayed that measurement and manipulation was impossible (Fig. 3.2G). At day 240 the control frog was nothing more than a dark organic sheet that draw the surface and contour of the animal (Fig. 3.2H), and the remains disconnected after slight movement. This advanced decay contrasts with frogs that stayed in mat (Fig. 3.2D): at this time the later preserved, for instance, complete articulation of limbs. The skin was not pierced, remained elastic and retained the original texture full of bumps (Fig. 3.3A and B). At a microscopic level, the tegument seemed to have a flatter surface after 540 days (1.5 years) (Fig. 3.3C), and was covered by a layer of small filamentous or slightly bacillary microorganisms (Fig. 3.3D). After day 1080 (3 years), the skin was still unbroken and present all over the bones (Fig. 3.3E). Its remarkable preservation allowed the observation of the underlying still-organized fatty tissue, where still-preserved adipocytes were depicted (Fig. 3.3F). In addition, other soft tissues were also preserved even after long time (540 and 1080 days). For instance, several fibres at the end of a bone, which are part of a joint, can be observed with SEM (Fig. 3.4A-C). At higher magnification, these filaments showed striping which is consistent with the presence of collagen in tendinous fibres (Fig. 3.4C). Additionally, the existence of other layers made by fibrous tissue can be detected (Fig. 3.4D-F); these layers would correspond to muscle, according to their appearance and layout. The good preservation of the skin and the presence of tendons and muscles even after 1080 days, and the protection afforded by the sarcophagus surrounding the bodies, allowed the preservation of fully articulated remains (Supplementary Fig. 3.3A-F).

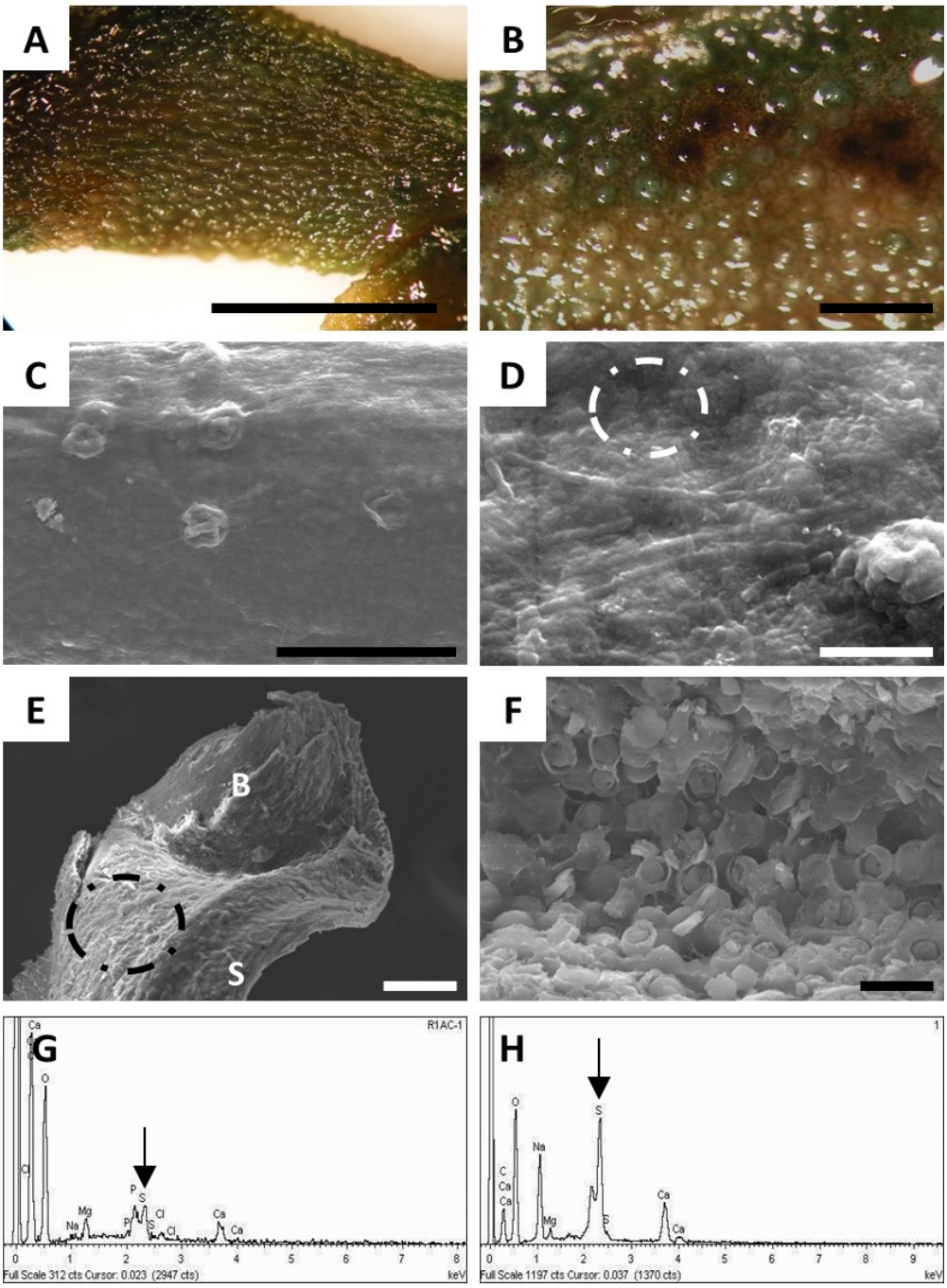


Fig. 3.3: Preservation of the skin of frogs inside the sarcophagus during the experiment. (**A**) Bumps of the skin from the back limb at day 120. (**B**) Bumps at day 240 observed at higher magnification and with SEM (**C**). (**D**) SEM image of a thin microbial veil made by cells and EPS covering the surface of the skin after 540 days. (**E**) Detail with SEM of the skin covering a bone at day 1080. (**F**) Adipocytes of the skin of a frog preserved inside the mat after 1080 days. (**G**) and (**H**) show the EDXS spetra recorded at the spots highlighted in (**D**) and (**E**). Black arrows point the pic corresponding to S. Scale bars represent 0,5 cm in **A**; 100 µm in **B**, **C** and **E**; 10 µm in **D** and **F**.

Bones belonging to frogs in mats were also remarkably preserved after 1.5 years (540 days, Fig. 3.5A and B) and even after 3 years (1080 days, Fig. 3.5C an D) while controls completely disappeared after that time. Sections of the bone showed the exceptional integrity of the mineralized tissue, which sometimes presented mesenchymal tissue

coating its inner face (Fig. 3.6). Other bones sections were filled by an amorphous organic residue that would likely correspond to the fatty marrow (Supplementary Fig. S3.4). Also especially noticeable was the conservation of eyes and brain (which could be seen through the skull) in frogs of 120 days (Fig.3.7). In fact, the sagittal section of the animal exhibited the presence of a white tissue inside the skull after 540 days (Fig. 3.8A) and 1080 days (Fig. 3.8D). According to the frog brain anatomy (Fig. 3.8A), the preserved brain part corresponded to the tectum (optic lobes), in the midbrain. The analysis with SEM of the inside of the frog confirmed that these brain structures have been mineralized, being transformed into calcium carbonate crystals (Fig. 3.8B and E and the corresponding EDXS spectra Fig. 3.8C and F).

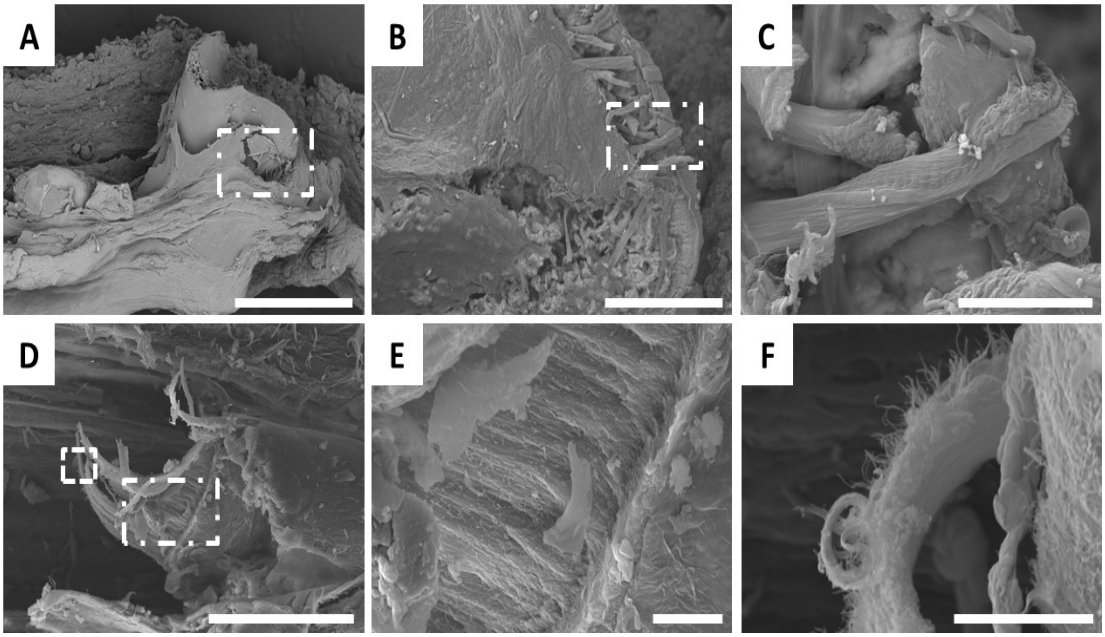


Fig. 3.4: Observation with SEM of soft tissue preservation in frogs covered by mat after day 1080. (**A**) Femoral head of a frog. (**B**) Zone highlighted in **A** at higher magnification, showing the presence of fibrous fibres corresponding with tendons, which are magnified in (**C**). (**D**) Layer of organic tissue still preserved ripped during the preparation of the sample showing a fibrous layer, likely muscle (**E**). (**F**) Detail of one of these muscular fibres highlighted in **B**. Scale bars represent 0,1 cm in **A**; 200 µm in **B**, 30 µm in **C**; 100 µm in **D** and; 10 µm in **E** and **F**.

Statistical Analysis

Tracking qualitative variables provided a detailed description of the decay pattern and the generation of a data matrix for the Hierarchical Cluster Test (Supplementary Table 3.1). The advanced decay of frogs on sediment (controls) did not allow data collection after day 45 because of specimens break down after manipulation. The resultant dendrogram (Fig. 3.9) showed two major clusters: one including samples from mat and the control at day 7 (Fig. 3.9 G1, light green), and others grouping controls from day 15 (Fig. 3.9 G2, dark green). Both were clearly defined according to decay state. The G1 branch is utterly divided into two other minor clusters. The first one (G1.1) grouped samples with none or subtle damages, explaining the inclusion of the day-7 control that

did not show remarkable decay yet. The second one (G1.2) included the rests of samples over mat. The last cluster (G2, red) was formed by controls, showing moderate to extensive decomposition. These groups were congruent with the outcomes of decay monitoring. Morphometric parameters showed that body size varied over time. Although frogs decaying on mats had an extensive loss of volume (up to 50% for thigh and abdomen thickness), statistical tests showed that this loss was significantly higher in controls. These differences rose from day 15 onwards. In the case of samples from mat tanks, the loss of volume seemed to correlate with the kind of variable measured, being higher in the case of thickness (T), moderate for width (W) and lowest for length (L) (plots in Supplementary Fig. S3.5).

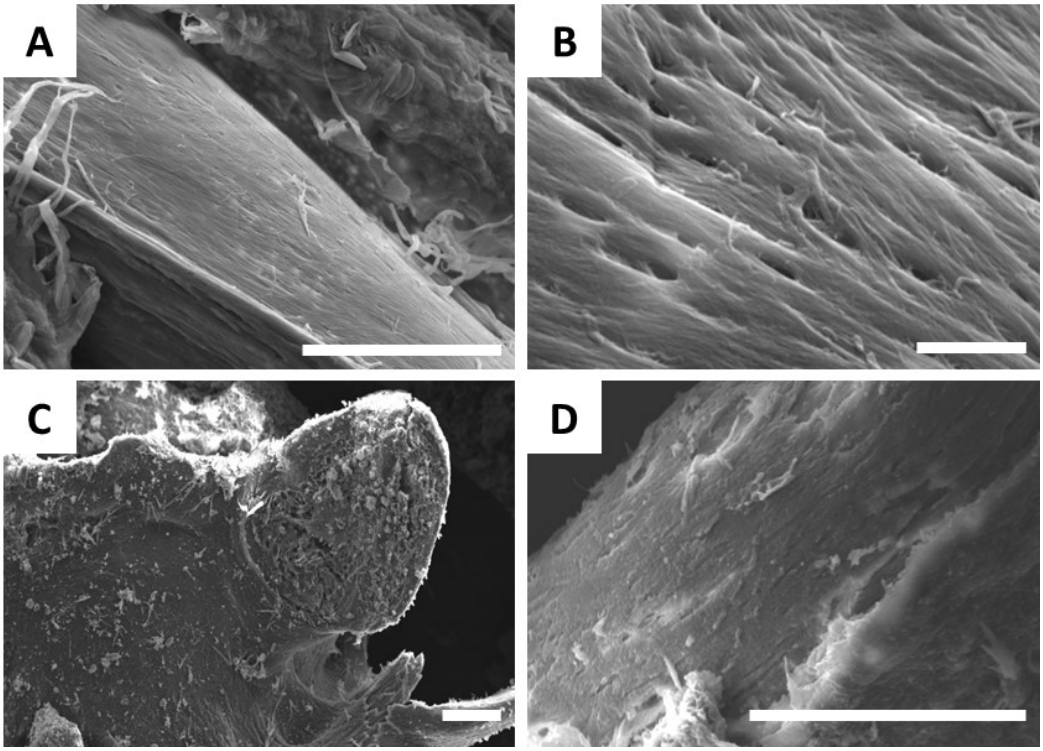


Fig. 3.5: Observation with SEM of the external state of bones in the course of the experiment. (**A**) Surface of a bone after 540 days. (**B**) Bone surface at higher magnification. (**C**) Section of a bone still covered by the skin at day 1080. Where the skin is absent, the surface of the bone can be observed (**D**) Detail of the surface. Scale bars represent 100 µm in **A**, **B** and **D** and 5 µm in **B**.

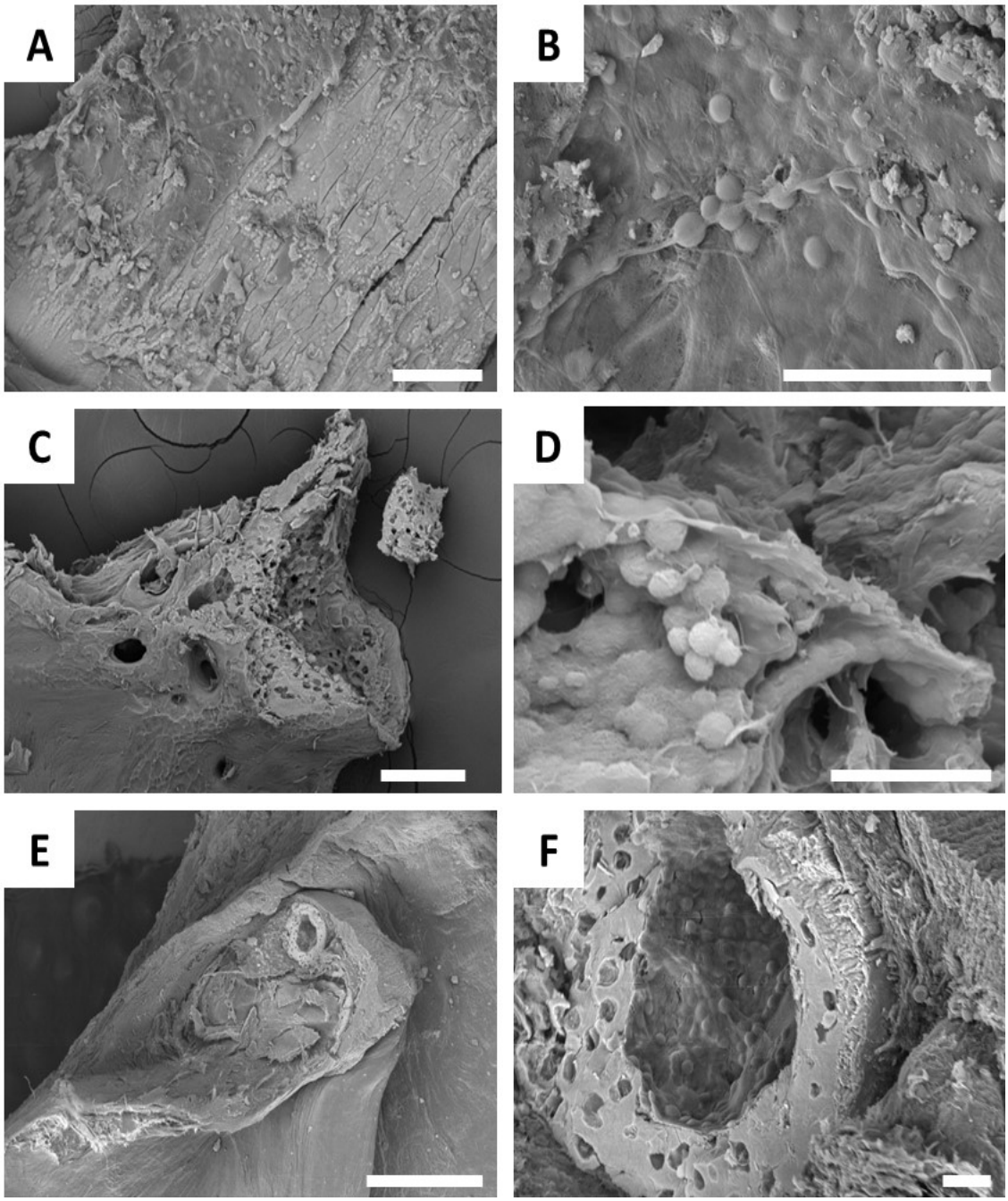


Fig. 3.6: Observation with SEM of the inner face of bones at day 540. (**A**) Inside of a broken bone with an organic coat in the inside face. (**B**) Detail of the organic coat. (**C**) Femoral head broken during prepration in order to exose the inside. (**D**) Detail of filling of the bone, where hematopoietic cells can be detected. (**E**) Section of a bone. (**F**) Hematopoyetic tissue inside the bone in **E** at higher magnification. Scale bars represent 200 µm in **A**; 50 µm in **B**, 100 µm in **C**; 10 µm in **D** and **F**: and 500 µm in **E**.

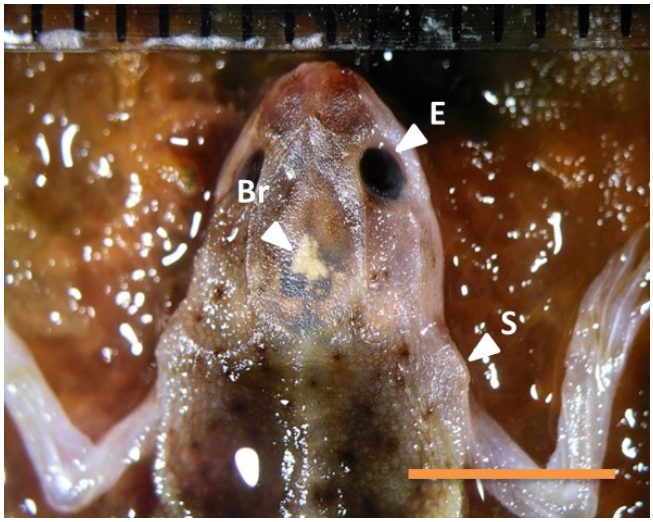


Fig. 3.7: Head of a frog after 120 inside the sarcophagus. The tegument and the bone have became slightly transparent and the brain (Br) can be observed through them. In addition, the eyes (E) are still preserved and the skin (s) is not pierced nor broken despite the time in the tank.

Scale bar represents 5 mm.

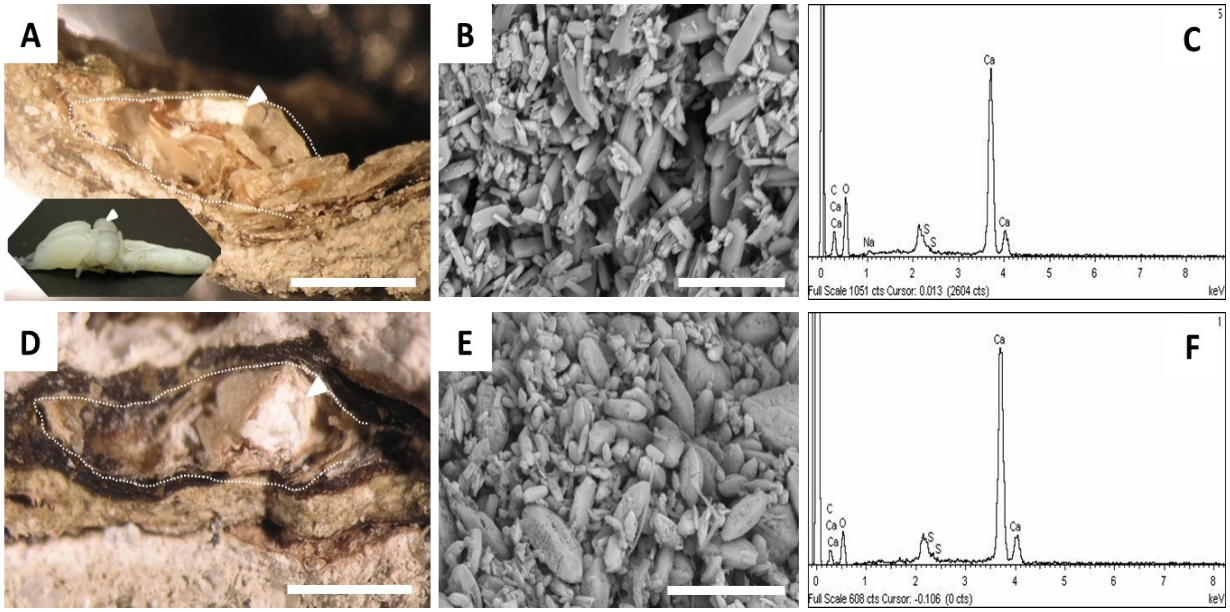


Fig. 3.8: Analisis of the brain preserved inside the skull of frogs preserved in the microbial sarcophagus. (**A**) Sagital section of a frog at day 540. The brain (arrow) can be detected even with binocular loupe. (**B**) Detail of the carbonates in the brain. (**C**) EDXS spectrum of the carbonate in **B.** (**D**) Sagital section of a frog inside the sarcophagus at day 1080. (**E**) Mineralized brain tissue (carbonates). (**F**) EDXS spectrum of minerals in **E**. Scale bars represent 0.5 cm in **A** and **D**, 20 µm in **B** and **E**.

DISCUSSION

The present experiment, based in a periodical observation of frog carcasses placed over microbial mats and sediments, allowed the description of the decay sequence. Bodies in control tanks showed a quick decomposition, starting with a loss of integrity from about day 30. These observations are vouched by the Hierarchical Cluster Analysis performed, which grouped separately frogs from control tanks and mats, meaning a significant level of dissimilarity. This is congruent with the results of a similar analysis performed with fish in mats, where samples were also clearly grouped according to the tank where they were placed (mat or sediment) (Iniesto et al. 2013). Differences between samples in mat and controls are also significant with regard to morphometric measures. Statistical Analysis showed that thigh thickness, total length and head size were strongly influenced by the presence of mats, avoiding its massive decrease. The reduction came after an early swelling of bodies, a typical forensic phase already described in taphonomic experiments, likely driven by the endogenous microbial decaying populations (Cambra-Moo et al. 2008). During this short period (7-15 days), carcasses on mats and controls suffered a similar decay process, explaining why the first control (day 7) was grouped with mat samples. After this early stage, controls began a quick decay that lead to complete disarticulation at day 40-45, although carcasses on mat remained articulated all along the experiment (1080 days). Previous works conducted with fish in microbial mats also showed a delay in decay (Iniesto et al. 2013, 2015a), with carcasses that persisted articulated after 5 years (Iniesto et al. 2015b).

In addition to the exceptional delay in decay recorded in frogs from mats, these experiments have showed several remarks that should be considered in order to understand the role of these microbial communities in preservation. As explained above, carcasses in mats were fully articulated all along the experiment while controls disconnected after a few weeks. Conserved articulation should be promoted by both the consistent sarcophagus that protects the bodies and the exceptional preservation of soft tissues. For instance, tendons and muscles were detected with SEM after more than 1 year. The present experiment depicts the organic preservation of tendinous fibers in the surroundings of bones. In addition, a fibrous tissue layer was also observed. According to previous observations (Briggs & Kear 1993), its organization and appearance is coherent with the presence of muscle. The morphology of these tissues is similar to organic remains preserved in fossil frogs from Libros, where fibers made by calcium phosphate have been observed (McNamara et al. 2009). In addition, the skin in our samples was not pierced or broken after 1080 days, contributing to the conservation of the articulation. Indeed, the state of preservation of the tegument was so remarkable that the original external morphology of bumps was maintained at 1.5 years. The existence of a cover made by small filamentous or slightly bacillary cells embedded in the EPS matrix, both present after 1.5 and 3 years, could also promote its conservation. In addition, the EDXS analysis of the surface of the skin showed an enrichment in sulphur, which in certain occasions would lead to the precipitation of calcium sulphate (likely gypsum) (see Fig. S3.3). Such increase in sulphur concentration would be related to the

occurrence of gypsum over the preserved skin of adult frogs in Libros (McNamara et al. 2009).

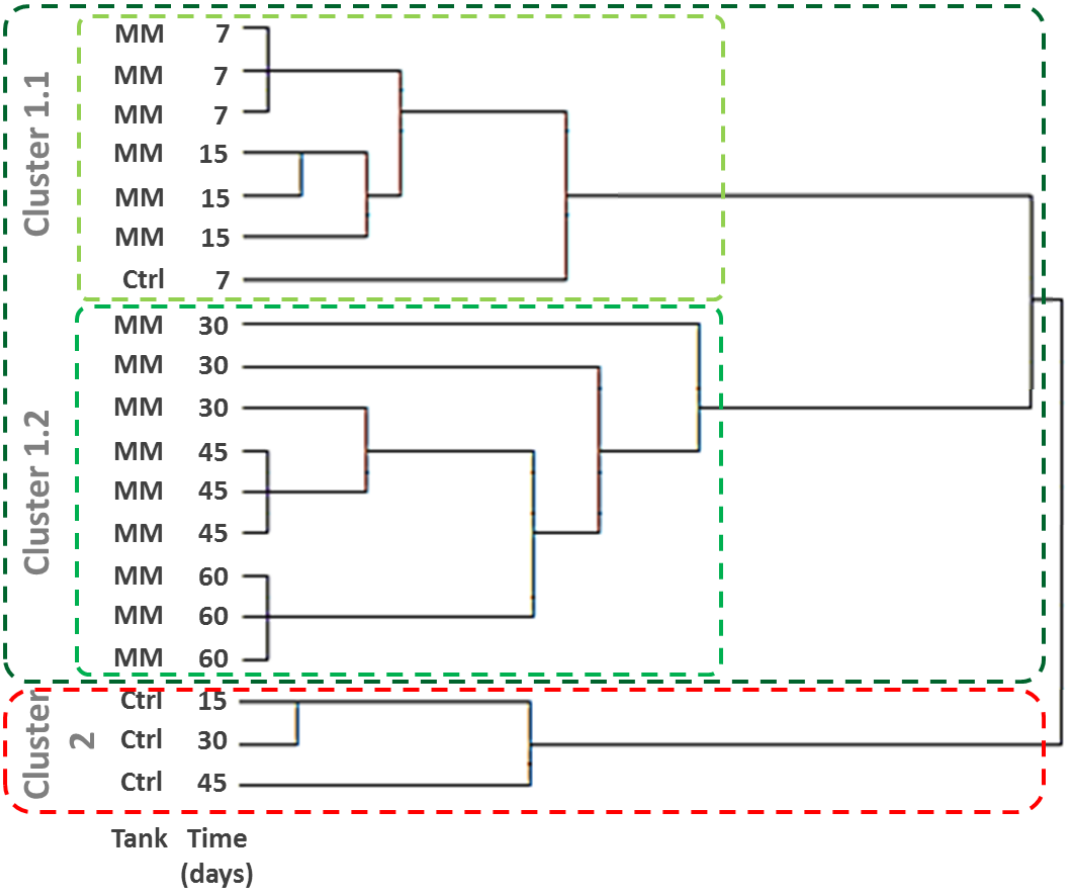


Fig. 3.9: Dendrogram showing the sequence of decay in frogs comparing carcasses over mat and controls over sediment. Initially, both, control and mat samples were swelled (first 7 days), although bodies in mat samples recovered slowly their thickness on day 15. The longer persistence of the swelling in mat samples was consequent with the state of the tegument. Controls presented at day 15 a loss of skin rugosity (likely pierced), quite disarticulation, and deformation, being impossible the accumulation of gases. During the swelling-time, the observation of the Linea alba was difficult. In addition, a thin heterotrophic veil grew rapidly over controls, supporting the statement of the active decay. Mat samples did not show any deformation nor disarticulation along the experiment, and a thick layer of mat covered the bodies

Another feature that allowed complete articulation of carcasses was the scarce damage suffered by bones. Observations with SEM showed that, even after 1080 days, they still had the original porosity and organization, made by mineralized tissue. Even more striking is the preservation of the marrow inside. Bone sections showed two different organic tissues which would correspond with the original bone marrow. Hematopoietic tissue coating the inner face of the bone was detected, as well as other samples showed an amorphous structure that would correspond with the fatty marrow. This kind of tissue is extremely labile (Custer 1974) and scarce in the fossil record.

However, bone marrow has been described in frogs from Libros (McNamara et al. 2006). In fact, the thorough analysis of amphibian from this site revealed that marrow was identified in 10% of bones of frogs (McNamara et al. 2006). Recently, Bertazzo et al. (2015) detected the occurrence of bloody cells, which were similar to those observed in the present experiment, inside non-exceptionally preserved bones of different therapods. These outcomes suggest that organic preservation of this type of tissue can be more frequent than expected (Bertazzo et al. 2015).

Although there is a considerable difference in preservation between frogs in controls and in microbial mats, the later also suffered decay, which was at the base of changes in environmental conditions in the tanks. Early decomposition leads to a drastic reduction in oxygen concentration and pH in the first 14 days in tanks with mats. This acid and reductive microenvironment has already been described in experiments with fish using microsensors (Iniesto et al. 2015a). The heterotrophic activity of microorganisms implicated in the decay consumed DO and acidified the system by the release of acid compounds during decay. The later rise of oxygen concentration and pH would be explained by the reduction of decomposition rates and the increase in photoautotrophic cyanobacterial growth favoured by nutrients released during decay. All this leaded to quick coverage of bodies of frogs in about 20 days and subsequent formation of the sarcophagus. Previous experiments testing decay and mineralization carried out with heterotrophic microbial communities have also shown a decrease in pH and DO. These environmental conditions have been linked to the occurrence of a mineralized coating of calcium carbonate (Martin et al. 2003) and/or calcium phosphate (Martin et al. 2005) in invertebrate eggs. Other assays performed with adult shrimps described a drop in pH which apparently inhibited the precipitation of calcium carbonate in favour of calcium phosphate in soft tissues (Briggs et al. 1993). These bioprecipitates occurred within one to four weeks from the beginning of the experiment and have been linked to microbial activity, which generates anoxic conditions and a decrease of pH. In our experiment, pH and DO were stabilized after seven weeks around the initial values and calcium phosphate precipitates have not been observed. However, we found that a part of the midbrain of frogs was turned into carbonate crystals after 540 days (1.5 years) and this mineralization remained after 1080 days (3 years). The occurrence of calcium carbonates can be explained by the saturation of the water in Ca and high pH. However, this precipitation is not widespread, and carbonate mineralization is restrained inside the skulls, in particular the tectum in the mid-brain. This spatial-limitation of the carbonate precipitation cannot be explained only by environmental conditions because pH and DO are homogenous inside and around carcasses as observed in experiments using microsensors (Iniesto et al. 2015a). Although those experiments were conducted with fish, the microenvironmental conditions were driven by microbial mat activity and is expectable that pH and DO is also similar in the case of frogs. In consequence, mineralization is not only explained by physico-chemical settings, but it should be also related with animal morphology and the original tissue composition. Indeed, differences in mineralization can reflect that several soft-tissues are more likely to be preserved (Wilby 1993). This statement is also supported by the fossil record since similar carbonate mineralization of the brains of frogs have been reported in tadpoles and adult

frogs from Libros (McNamara et al. 2009; 2010) and from Las Hoyas (Buscalioni, personal communication). In addition, mineralization of soft tissue other than brain was also depicted after 4 years in the case of fish inside mats, where a Mg-rich silicate phase was detected replacing bones and several inner organs (Iniesto et al. 2015b). On the contrary as happened in experiments with heterotrophic biofilms, no calcium phosphate phases have been reported in our assays with mats. Differences observed in mineral precipitation between experiments with mats and those performed with simple biofilms can be explained by differences in metabolic activities. In absence of mats, chemical conditions for mineralization are generated by heterotrophic decaying populations (Briggs 2003), thus the process had to be rapid in order to preserve some organic tissues. In presence of mats, microenvironmental conditions are mostly determined by the upper layers, i.e. autotrophic photosynthetic populations, decay is significantly delayed and, in consequence, it is not necessary that mineralization occurs quickly. On the other hand, it has been broadly accepted that acidity and anoxia are necessary to calcium phosphate precipitation (Briggs and Wilby 1996). However, this mineral phase can occur even at high pH (Song et al. 2002) if the solution is saturated in phosphate (Recillas et al. 2012). In consequence, differences in pH values do not explain by themselves the phosphatization of tissues. This kind of mineralization may be explained by other factors such as intensity of decay (slowed-down or active) or type of decaying tissue.

Exceptional preservation by microbial mat populations can be achieved by organic preservation and authigenic mineralization as exposed in the present paper. Previous experiments have already established the impact of the presence of microbial mats in fish decay through experiments based on morphometric data and long term monitoring (Iniesto et al. 2013), microsensors measurement of the microenvironment (Iniesto et al. 2015a), and bioprecipitation checking (Iniesto et al. 2015b). The present work connects these results with the decay of other vertebrate group. In this case, the presence of limbs makes these results more transposable to explain taphonomic processes in other groups of vertebrates such as mammals or reptiles. The preservation of the thin tegument of frogs observed in presence of mats would be enhanced in the case of carcasses with harder skin. Indeed, the intimate contact between consistent teguments preserved into the sarcophagus and mats would generate actual imprints, preserving the external shape of extant animals such as dinosaurs (Platt and Hasiotis 2006). In addition, the longer the carcass stands preserved and articulated, the more time for bioprecipitation and mineralization will be available. Exceptional fossils of frogs collected in Las Hoyas (Lower Cretaceous, Iberian Range, Spain) showed such level of integrity that it was possible to perform a taxonomic study of the different samples (Báez 2013). Therefore, these experiments are capital to understand how exceptional remains were formed and to explain taphonomic processes leading to fossils collected nowadays. Experiments describing the sequence of decay and the loss of the articulation of limbed carcasses are reliable information for palaeontology. This is why longer experiments with mats should be conducted in order to establish a complete decay sequence including the formation and localization of different authigenic mineral phases.

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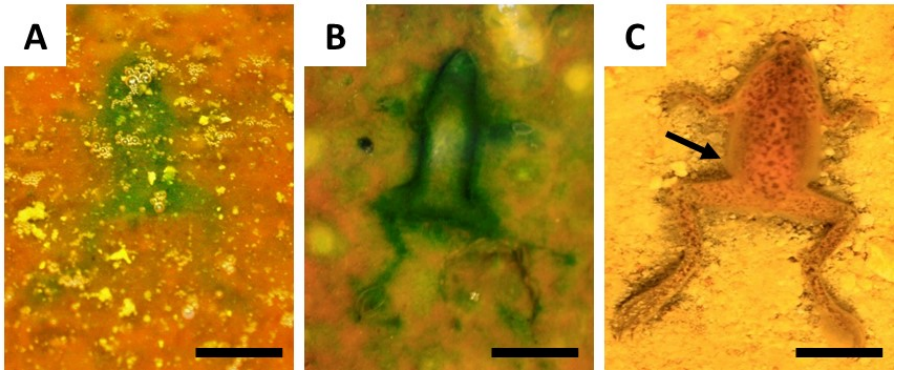
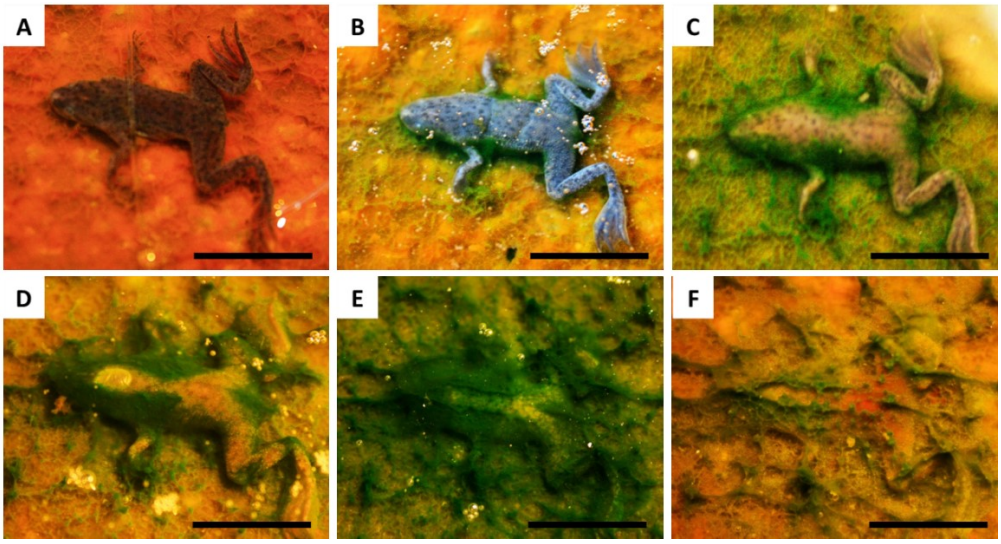
SUPPLEMENTARY FIG S3.1



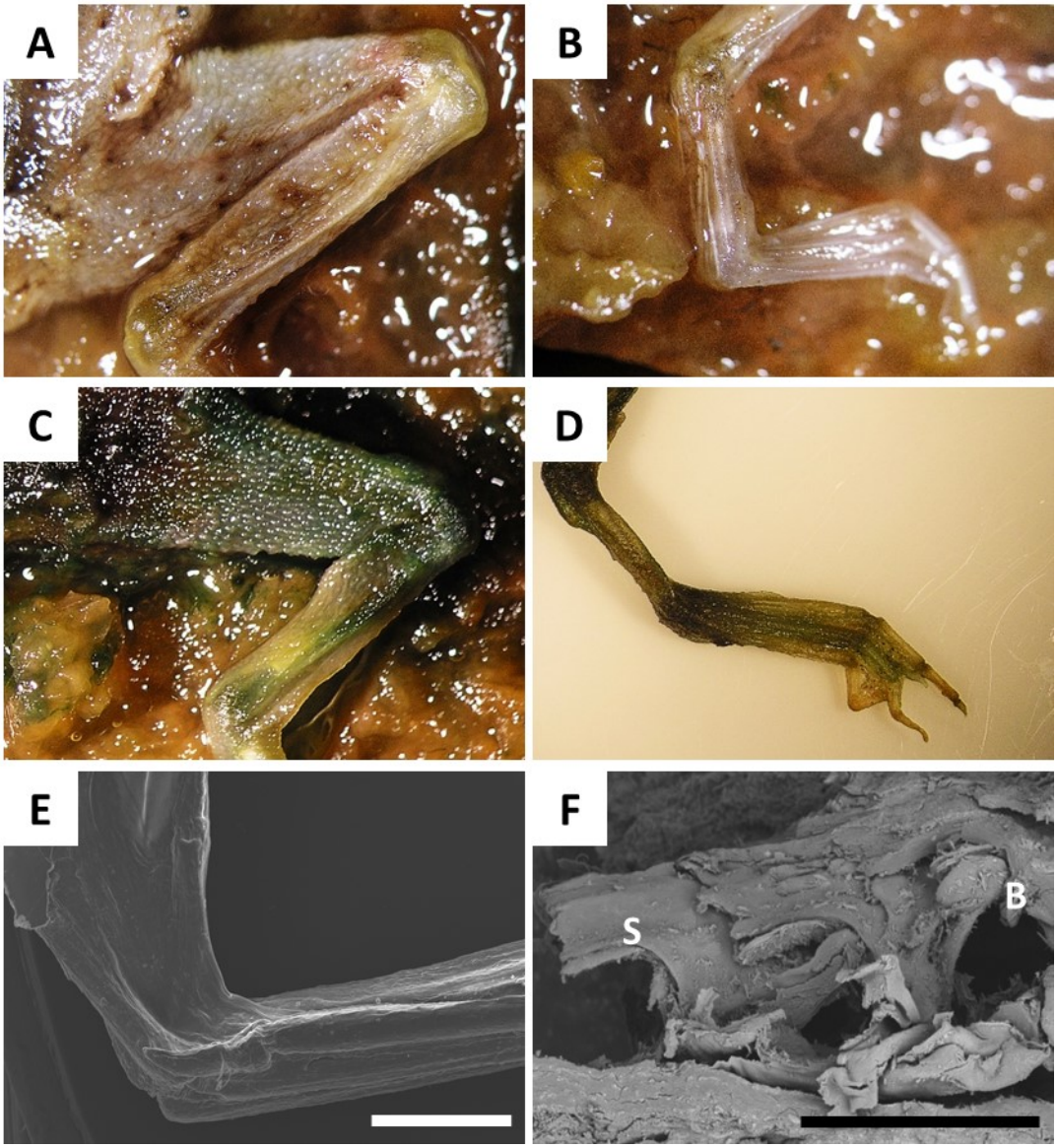
Supplementary Fig. S3.1: Effect of the placement of frog carcasses over mat (**A,** day 3 and **B**, day 7) or sediment (**C**). (A) The local stimulation of the mat lead to the drawing of the shape of the frog in the suface. (**B**) Despite the frog is not yet completely covered, at day 7 the mat begins to form the sarcophagus all over the body. The arrow in **C** shows the darkening of the surface of the sediment in direct contact with the body. Scale bars represent 1 cm.

SUPPLEMENTARY FIG S3.2



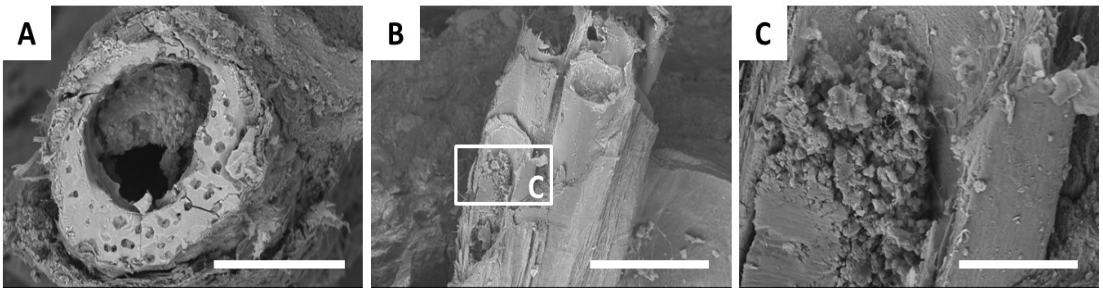
Supplementary Fig. S3.2: Sarcophagus formation and coverage of frog carcasses by mats. (**A**) Frog over the mat at the beginning of the experiment. (**B**) Frog at day 12, with a distinguishable green-darkening under the carcass. (**C**) Frog partially covered at day 17. (**D**) Frog completely trapped by mat at day 28. (**E**) Thick sarcophagus covering the frog at day 43. (**F**) At day 60, the frog is embedded in the microbial community. Scale bars represent 1 cm.

SUPPLEMENTARY FIG S3.3



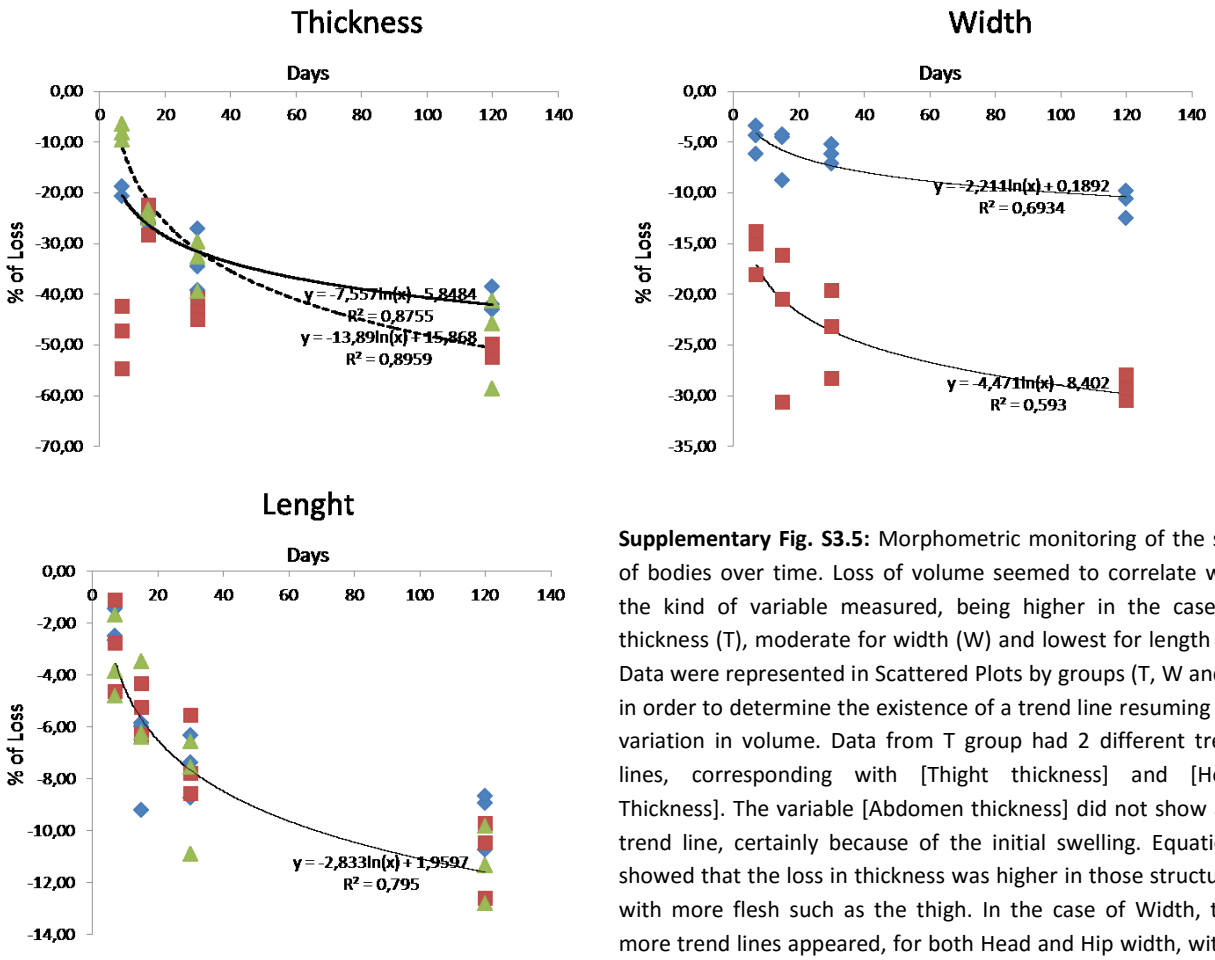
Supplementary Fig. S3.3: State of articulation and soft tissue preservation of *Hymenochirus boettgeri* inside the microbial sarcophagus. (**A**) Knee of a frog after 120 days in the mat. (**B**) Detail of the foot at day 120, where nails are still preserved. (**C**) Knee at day 240. (**D**) Foot of the frog at day 240 with nails still present. (**E**) Elbow of the frog at day 540 oberved with SEM. The skin is still preserved. Its smoothness let the detection of muscle and tendons beneath it. (**F**) Section of completely articulated vertebrates with the skin (S) covering bones (B). Scale bars represent 1 cm.

SUPPLEMENTARY FIG S3.4



Supplementary Fig. S3.4: Sections of frog bone exhibiting the exceptional preservation of the fatty marrow at days 540 (**A**) and 1080 (**B** and **C**). Images obtained with SEM. Scale bars represent 200 µm in **A**, 500 µm in **B** and 100 µm in **C**.

SUPPLEMENTARY FIG S3.5



Supplementary Fig. S3.5: Morphometric monitoring of the size of bodies over time. Loss of volume seemed to correlate with the kind of variable measured, being higher in the case of thickness (T), moderate for width (W) and lowest for length (L). Data were represented in Scattered Plots by groups (T, W and L) in order to determine the existence of a trend line resuming the variation in volume. Data from T group had 2 different trend lines, corresponding with [Thight thickness] and [Head Thickness]. The variable [Abdomen thickness] did not show any trend line, certainly because of the initial swelling. Equations showed that the loss in thickness was higher in those structures with more flesh such as the thigh. In the case of Width, two more trend lines appeared, for both Head and Hip width, with a smaller reduction in volume in those structures made mainly by bone such as the head (blue line). Finally, the group L showed a more stable loss, and one single trend line summarized the three variables measured [Femur], [Fibula] and [Total Body]. For lengths, the reduction was smaller, most likely due to the bones that are less responsive to decay.

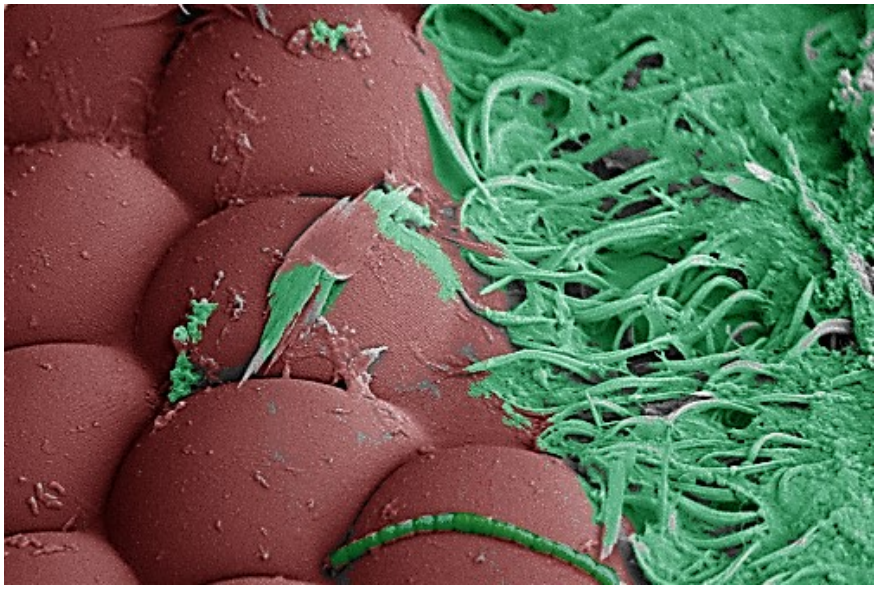
SUPPLEMENTARY TABLE S3.1

Supplementary Table S3.1: Sequence of changes observed during the process of decomposition of fishes in nonmat control sediment and tanks with microbial mats.Initially, both, control and mat samples were swelled (first 7 days), although bodies in mat samples recovered slowly their thickness on day 15. The longer persistence of the swelling in mat samples was consequent with the state of the tegument. Controls presented at day 15 a loss of skin rugosity (likely pierced), quite disarticulation, and deformation, being impossible the accumulation of gases. During the swelling-time, the observation of the Linea alba was difficult. In addition, a thin heterotrophic veil grew rapidly over controls, supporting the statement of the active decay (Fig. 3). Mat samples did not show any deformation nor disarticulation along the experiment (Fig. 3), and a thick layer of mat covered the bodies0 = no changes observed; 1 = minor changes observed (i.e., some rays of the fin are broken); 2 = moderate decay; 3 = major decay observed).

Time (days)	Tank	Swelling	Colour	Red colour	Linea alba	EPS	Skin
7	T1	3	0	3	2	0	3
7	T2	3	0	3	2	0	3
7	T3	3	0	3	2	0	3
7	C	3	0	3	2	2	3
15	T1	2	0	2	3	1	2
15	T2	3	0	2	3	1	3
15	T3	2	0	1	2	1	2
15	C	0	0	1	1	3	1
30	T1	0	0	0	1	2	0
30	T2	3	0	0	3	3	3
30	T3	0	0	0	1	2	0
30	C	0	0	0	0	3	0
120	T1	0	0	1	3	3	0
120	T2	0	0	0	3	3	0
120	T3	0	0	0	3	3	0
120	C	0	0	0	3	3	0
242	T1	0	3	0	3	3	0
242	T2	0	3	0	3	3	0

CAPÍTULO 4

INVOLVEMENT OF MICROBIAL MATS IN EARLY FOSSILIZATION BY FORMATION OF IMPRESSIONS AND REPLICAS OF VERTEBRATE AND INVERTEBRATE BODIES



Artículo en revisión en Scientific Reports:

Iniesto M, Buscalioni AD, Guerrero MC, Benzerara K, Moreira D and López-Archilla AI. Exceptional preservation of vertebrates and invertebrates mediated by the formation of a sarcophagus and the body impression in microbial mats

ABTRACT

Microbial mats have been hypothesized to improve the preservation of organic remains in the fossil record. We have tested this hypothesis with long-term experiments (up to 5.5 years) using both invertebrate and vertebrate corpses. Once carcasses are placed on mats, the microbial community coats the corpses and forms a three-dimensional sarcophagus composed of microbial cells and exopolymeric substances (EPS). This coverage provides a template for i) moulding superficial features, resulting in negative impressions, and ii) generating replicas. The impressions of fly setulae, fish scales and frog skin verrucae are shaped mainly by small cells embedded in a thick EPS matrix. Microbial cells also replicated delicate internal structures such as the three successive layers that compose a fish eye. The sarcophagus protects the body integrity, allowing inner organs preservation such as ovaries and digestive apparatus in flies, the swim bladder and muscles in fish, and the bone marrow in frog legs. This study brings strong experimental evidence to the idea that mats favour metazoan fossilization by moulding, replicating and delaying decay. Obrution has classically been invoked as the main mechanism to explain exceptional preservation. However, mats provide an interesting alternative as they can preserve complex features for a long time.

Keywords: exceptional preservation; experimental taphonomy; microbial sarcophagus; microbial entombment; moulds; soft-tissue preservation

RESUMEN

La presencia de tapetes microbianos ha sido con frecuencia considerada como un factor que favorecería la preservación excepcional en el registro fósil. El presente artículo pretende aportar una base experimental a esta hipótesis mediante un estudio llevado a cabo durante 5.5 años empleando cadáveres de vertebrados (ranas y peces) e invertebrados (moscas). Se comprobó que tras la colocación de los restos animales en la superficie de los tapetes, la comunidad microbiana los iba envolviendo, formando un sarcófago tridimensional compuesto por células microbianas y sustancias exopoliméricas (EPS). Esta cubierta sirvió de base para la formación de moldes en negativo de estructuras superficiales y para la generación de réplicas. Las impresiones de las sétulas de mosca, las escamas de pez y las protuberancias del tegumento de ranas estaban mayoritariamente formadas por células de pequeño tamaño embebidas en una fina matriz de EPS. Las células microbianas también replicaron estructuras lábiles como las diferentes capas que componen el ojo del pez. El sarcófago favoreció el mantenimiento de la integridad de los cuerpos, permitiendo además la preservación de órganos internos como el aparato digestivo y los ovarios en el caso de las moscas, la vejiga natatoria y músculos en peces o la médula ósea en ranas. Este estudio aporta una sólida evidencia experimental que confirma la idea de que los tapetes favorecen la fosilización a través de la formación de moldes, la replicación y el retraso de la descomposición de los cadáveres. El enterramiento rápido ha sido considerado comúnmente como uno de los factores principales para la preservación excepcional. Sin embargo, los tapetes microbianos son una interesante alternativa, pudiendo preservar estructuras complejas durante largos periodos.

Keywords: moldes; preservación excepcional; tafonomía experimental; tejidos blandos preservados; sarcófago microbiano

INTRODUCTION

A wealth of taphonomic studies has shown that processes enabling fossilization are uncommon, especially for soft tissues (Briggs 2003a, Sansom et al. 2010). Based on the frequent association between microbial biofilms, articulated fossils and mineralized soft tissues in many deposits like Konservat Lagerstätten, it has been suggested that microorganisms are an important factor for exceptional preservation (Briggs 2003b, Seilacher et al. 1985). This suggestion is highly supported by the thorough observation and analysis of the fossil record (Behrensmeyer 2001, Briggs 2003b, Buscalioni and Fregenal-Martínez 2010, Gall 1990, Gall et al. 1985, Martínez-Delclòs et al. 2004, O'Brien et al. 2008, Pawlowska et al. 2012, Seilacher 1990) and by several taphonomic studies, in which the presence of microbes positively affects preservation during the early diagenesis (Raff et al. 2008, Sagemann et al. 1999). The experimental contributions made in this field have focused on characterizing the processes that microorganisms prompt in the preservation of organic remains (i.e., capture, protection, inhibition of decay, mineralization and pseudomorphing (as summarized by Briggs, 2003a)), and the microenvironmental conditions necessary for these processes to occur (Sagemann et al. 1999). Most of these experimental approaches conducted so far have used heterotrophic bacteria and/or biofilms inoculated in different media (e.g. Briggs y Crowther 2003, Raff et al. 2008, Sagemann et al. 1999). In contrast, only few taphonomical experiments have used natural microbial mats. It is important to notice that biofilms present remarkable differences with microbial mats. In particular, biofilms have a simpler biological composition with a relatively small number of microbial species, whereas mats are complex communities, frequently comprising hundreds of species including photosynthetic oxygenic cyanobacteria, red and green anoxygenic photosynthetic sulphur bacteria, and a large variety of heterotrophic bacteria. As a result, mats develop a variety of metabolic reactions, some of them producing steep chemical gradients which are not as well developed in biofilms. In addition, mats contain multiple trophic levels (i.e., primary producers, consumers, decomposers) and can be considered as genuine ecosystems (Stolz 2000). Therefore, the outcomes of taphonomy experiments may likely be very different depending on whether biofilms or mats are used. For example, Darroch et al. (2012) tested the implication of microbial mats in the genesis of death mask favouring the preservation of Ediacaran fossils. Moreover, Iniesto et al.(2013) and Guerrero et al. (in press) observed the formation of a sarcophagus that covered carcasses, a remarkable slow-down in the decay, and the development of different microenvironments around carcasses (Iniesto et al. 2015a) showing the impact of microbial mats on early fossilization. Nevertheless, the comprehension of the mechanisms and processes leading to exceptional preservation requires more experimentation.

Moulds and replicas are widespread fossilization products. Gehling (1999) postulated the important role of microbial mats in the preservation of impressions as a fossilization pathway, by forming a "death mask" that maintained the shape of the specimen. In the formation of a mould, a negative copy of the external body parts (i.e., an impression) is

generated. The implication of microorganisms in mould formation has been suggested by numerous observations of fossils: microbial mats might be involved for instance in the preservation of the skin of dinosaurs (Briggs et al. 1997, Kim et al. 2010), in preserving body-shape, integument tissues and colour patterns (Rose 2012), in the formation of the skin shadow in ichthyosaurs (Martill 1987), and even in the preservation of ancient human footprints (Marty et al. 2009). This process was also crucial for the conservation of soft-bodied organisms (Gehling 1999, Narbonne 2005), where mats cemented the sediment around bodies, preserving the external morphology by authigenic mineralization (Darroch et al. 2012, Laflamme et al. 2011, Meyer et al. 2014). In contrast, replication results from intensive microbial colonization that reproduces the original tissues, followed by the replacement and stabilization of the fossil within the sediment (i.e., replica). However, preservation through replication by microbes has rarely been described and is limited to few examples, such as the endoreplica produced during the coating process, replicating pliable vascular structures and osteocytes in bones (Kaye et al. 2008). Another remarkable example comes from experimental embryo fossilization by thin heterotrophic biofilms, which replaced and replicated the morphology of the consumed embryo (Raff et al. 2008).

In contrast with simple biofilms, microbial mats are organized in layers and each one has a specific community composition and metabolic functions (Cohen and Rosenberg 1989, Dupraz and Visscher 2005, Dupraz et al. 2009). According to microenvironmental conditions previously described (Iniesto et al. 2015a), the deepest layers are anoxic. The upper layers would be associated, because of the formation of a dense sarcophagus, with the delay in decay and the protection against abiotic factors such as water flow (Briggs 2003b, Buscalioni y Fregenal-Martínez 2010). Furthermore, mineral precipitation may occur in all layers. Indeed, Schiffbauer et al. (2014) showed that bacterial sulphate reduction, a metabolism that is present typically in deep layers, can induce the pyritization of fossils, while Iniesto et al. (2015b) evidenced the formation of Mg-rich silicates within the green upper layer of an experimental mat fossilizing fish. The present study aims at understanding the involvement of microbial mats in animal body preservation through the monitoring of changes that occurred during decay of carcasses. It focuses specifically on the phototrophic oxic layer (top level of the mat), showing that its intrinsic properties and functions are critical in the tapho-generation of moulds and replicas within a microbial sarcophagus. The tested taphonomic phase extends from the subaerial exposure of a body to its complete coverage by the microbial mat, prior to burial within the sediment. We herein describe long-term experiments, up to 5.5 years, using flies (*Musca domestica*), fish (*Carassius auratus* and *Paracheirodon innesi*) and frogs (*Hymenochirus boettgeri*). Carcasses were placed in tanks with microbial mats to observe the formation of imprints and replicas. We imaged inner soft-tissues to assess the preservation and the potential invasion of the microbial mat within the animal bodies.

MATERIAL AND METHODS

Original mat samples were taken at Lake Salada de Chiprana (Zaragoza, Spain), which is a hypersaline (30–70‰) permanent shallow lake of endorheic origin in a semi-arid region of the Ebro depression (Aragon, NE Spain) (Guerrero et al. 1991). The lake is fed by ground water discharge, which provides a source of abundant magnesium sulphate (up to 700 meq.L^{-1} of SO_4^{2-} and Mg^{2+}) and sodium chloride (approximately 300 meq.L^{-1}). The mats from the Salada de Chiprana are dominated by the filamentous cyanobacterium *Coleofasciculus chthonoplastes* (Thuret ex Gomont) M. (Siegesmund et al. 2008) (formerly known as *Microcoleus chthonoplastes* Thuret ex Gomont), which forms the framework of the mats. The upper layer of the mat is mainly formed by the taxa Chloroflexus, Coleofasciculus and Pseudoanabaena-like, while the red layer below is composed of anoxygenic photosynthetic bacteria, and the dark layer in the bottom is made up of anaerobic microbial populations and dead cyanobacteria (Jonkers et al. 2003). Chiprana mats were prepared as described in previous experiments (Iniesto et al. 2013) and placed in three 50x25x20 cm glass tanks containing a 2–3 cm base of limestone overlain by a 3–4 cm layer of sediment from the lake, and grown in the laboratory. Tanks were also filled with water from the lake in order to get a 2 cm water column (details of the ionic water composition can be checked at Iniesto et al. 2015b). Chambers were illuminated with a constant light output and a cold beam (OSRAM Decostar 51 Titan) with a photoperiod similar to the natural one (adjusted to 10 h of daylight). Three additional tanks, used as controls, were prepared following the same procedure but without the microbial mat overlaying the sediment. Control tanks were kept in dark in order to avoid the development of a microbial mat from the resting stages of photosynthetic populations present in the sediment. All the tanks were placed in a controlled-temperature room. During the experiment, temperature in tanks was 23 ± 0.5ºC, and pH was 8.6 ± 0.7 in tanks with mats and 8.3 ±0.5 in non-mat tanks. Conductivity (50.1 ± 2.3 mS·cm^{-1} and 52.2 ± 1.8 mS·cm^{-1} for mat and non-mat tanks, respectively) and water depth (about 2 cm) were kept almost constant in tanks by the periodical addition of sterilized distilled water to compensate for water evaporation.

Three groups of animals were tested in order to identify possible differences in mould formation: one arthropod (fly) and two vertebrates (fish and anuran). The arthropod was *Musca domestica* (grown in the laboratory from wild specimens). Fish species were the red carp (*Carassius auratus*) and the neon tetra fish (*Paracheirodon innesi*) and the anuran was the “nailed small toad” (*Hymenochirus boettgeri*). All animals were euthanized with tricaine mesylate (MS-222, 0.06%) diluted in TRIS (0.29%) following the Animal Care Protocol of the Universidad Autónoma de Madrid (U.A.M.). All the experimental protocol was checked and approved by the authorized body of the government of the Comunidad de Madrid (Clinical Research Ethics Committee of the U.A.M.: http://www.uam.es/otros/ceiuam/), which evaluates protocols and projects that include experiments with animal. Carcasses were laid on the surface of microbial mats. Flies, fish and frogs were placed in separated tanks. Control carcasses were placed

in independent tanks without mat. The microbial covering process was monitored over the whole experiment time. According to previous experiments, bodies were distributed on the mat surface with a lateral distance of 4 cm in order to avoid interferences between them. To examine the progress of mould formation, arthropod samples were examined at two times: 8 and 66 months (5.5 years). The red carps were observed at month 15, neon tetra fish at months 8 and 24, and anurans at months 8 and 12. Two specimens were studied for each species at each time. To observe these specimens already embedded inside the microbial community, blocks of the mats containing the animal bodies were extracted from the tanks. When the mats were easily detachable from the bodies, the microbial cover was removed to observe the carcass surface and the microbial veil in direct contact with it. Blocks were also cut at the centre to get two parts where the carcass was exposed. One of the parts was emptied by removing the carcass in order to analyse the sarcophagus generated by the mat and the imprint formation. The other part allowed the observation of the relation between the mat coverage and the carcass as well as the inner tissue preservation. In every case, a first observation of carcasses and moulds with a binocular magnifier was carried out. More detailed observations were achieved using Scanning Electron Microscopy (SEM). Because of the inherent features of the mat, in particular its dense EPS layer, chemicals used in sample preparation have difficulties to penetrate inside the block; this is why, after previous trials carried out in our laboratory, a specific protocol for microbial mats was established. Following these trials, fly, fish, and anuran samples were fixed in vacuum at room temperature during 64 h with 2% glutaraldehyde and dehydrated in ethanol at increasing concentrations (30%, 50%, 70%, 90%, 3x100%, 60 min each step) and dried overnight at 37ºC. To obtain precise details, several samples were embedded in epoxy resin and polished. Samples for SEM observation were coated with carbon or gold to increase electron conductivity. Observations were performed using a Zeiss Ultra 55 SEM equipped with a field emission gun and a Hitachi S-300N. Images were acquired with the microscopes operating at 15 kV and a working distance of ~7.5 mm and 15 mm, respectively. Several anuran samples (8 months) were also observed using an environmental SEM FEI QUANTA 200. In this case, fixation was not necessary. The size of cells observed by SEM was analysed afterwards with the imaging software Cell-A 3.0. In order to monitor the conservation state of inner tissues, T2-weighted MRI, performed with a Bruker BMT 47/40 MRI scanner, allowed the observation of several fish bodies prior to fixation and resin embedding. MRI is a non-destructive technique that provides good contrast between the body parts and tissues.

RESULTS

Formation of sarcophagi

Fly, fish and frog bodies were placed both on the surface of microbial mats grown in tanks and on the surface of sediment in the control experiments. Animals were completely covered by mat microbes after one to three weeks depending on the type and size of the animal (the smaller the size, the faster the coverage; see Supplementary Fig. S4.1 for a detailed summary of the sequence of coverage and preservation in mats). Despite these variations in duration, the process always followed the same pattern. After carcasses were laid on mats, cyanobacterial filaments from the upper layer of the microbial community started to grow on the bodies and trapped them (Fig. 4.1). After this stage, an increasingly thick veil grew over the animals and formed the final three-dimensional sarcophagus (Fig. 4.1C-D, 4.1G and 4.1J). Scanning electron microscopy (SEM) observations allowed describing the details of this coating process and the occurrence of abundant exopolymeric substances (EPS) produced by the microbial mats (Fig. 4.2). The dense and consistent EPS-rich matrix was always embedding the different microbial cells observed in the course of the experiment, being a constitutive element of the developing sarcophagus. In the case of flies, the microbial veil developed as a sheath over the carcasses, including wings (Fig. 4.2A) and legs (Fig. 4.2B). A consistent coat was observed over fish bodies (Fig. 4.2C shows a detail around the fish tail region). The removal of the microbial coverage allowed the observation of fish spines also coated by a film of coccoid-shaped bacteria at month 15 (Fig. 4.2D). Similarly, after the removal of the top layer, the complete articulation of frog legs could be observed (Fig. 4.2E). On the frog skin, which remained remarkably preserved even after 12 months (Fig. 4.2F), the presence of few coccoid and filamentous bacterial cells was detected.

After the full coverage of the bodies by the mat microorganisms was achieved, the microbial layer continued to grow until becoming a dense sarcophagus that reproduced the three-dimensional shape of the body. During this process, the size of the head and thorax of the flies remained constant within the sarcophagus, whereas the softer abdomen flattened over time (Fig. 4.1C and 1D). The fish bodies, covered by scales, were also quite resistant against flattening (Fig. 4.1G) but frogs showed obvious reduction of body thickness (Fig. 4.1J).

Formation of imprints and replicas

The microbial mats formed a negative print of the surface morphology of bodies, i.e., an impression, with high fidelity even at very fine scale. Fly exocuticles, which contain many hairs of different size and morphology and were embedded within the biofilm covering the bodies provided one particularly outstanding example (Fig. 4.3A-C). Microbial sheaths that surrounded the setae (biggest hairs) remained intact after the removal of the corpse (Fig. 4.3B) and several regions of the mat presented a multitude

of small impressions and holes corresponding to the setulae (hairs smaller than setae) (Fig. 4.3C). Even very delicate structures, such as wings, left an impression preserved by the mat. Not only the wing shape, but also the relief of the venation pattern (Fig. 4.3D) and the wing hairiness (Fig. 4.3E) were copied.

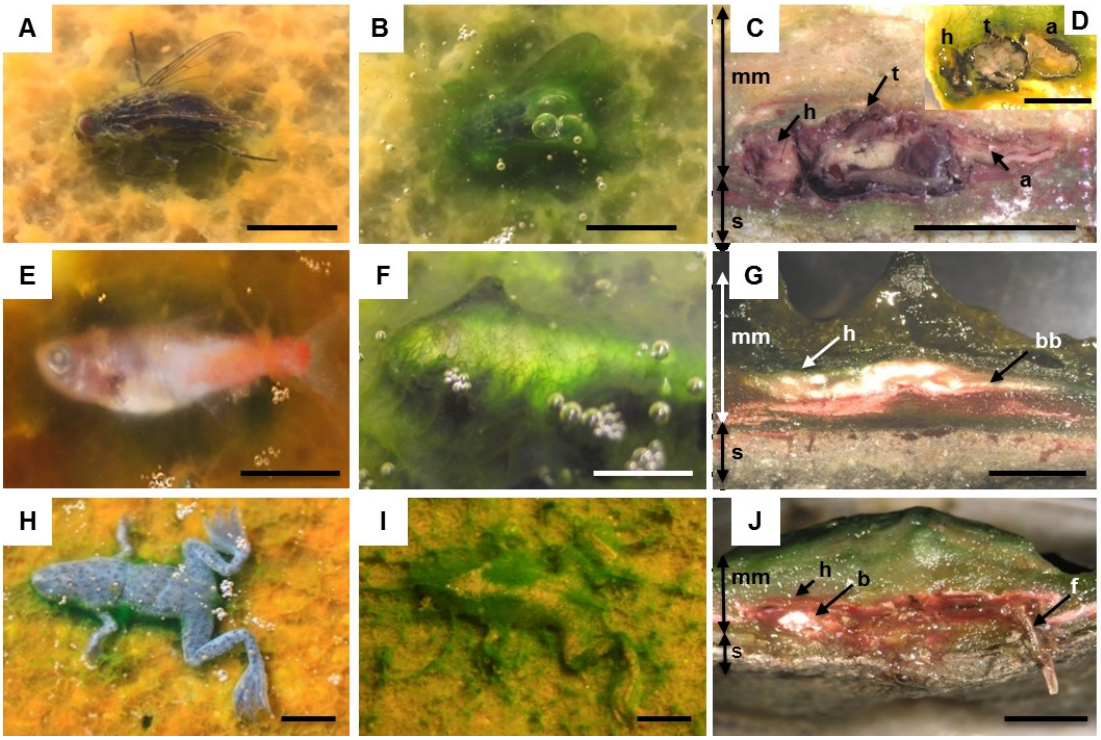


Fig. 4.1: Temporal sequence of the microbial coating of carcasses. (**A-D**) Fly (*Musca domestica*); (**E-G**) Fish (*Paracheirodon innesi*), (**H-J**) Frog (*Hymenochirus boettgeri*). The left column shows the organisms after one week on the mat. The central column corresponds to carcasses completely covered at day 19. The right column shows the sections cut across microbial mat blocks containing the carcasses. Note the coherence of the sarcophagus around the bodies of flies (**C,** 5.5 years and **D**, 8 months), fish (**G**, 8 months) and frog (J, 12 months). Abbreviations: a, abdomen; b, brain; bb, back bones; h, head; f, femur; mm, microbial mat; s, sediment; t, thorax. (Scale bars: A-D, 5 mm; E-J, 10 mm).

Fish bodies were also printed on the mats. The head impression clearly highlighted the eye and lens surfaces (Fig. 4.4A) and, in both cases, the printing showed fine microbial filamentous and slightly bacillary cells in an EPS matrix (arrow, Fig. 4.4B and 4.4C, respectively). By contrast, the top of the mat which was not in direct contact with the fish body showed a messy package of large filaments of the cyanobacterium *Coleofasciculus* (Fig. 4.4D). Scales were also printed on the mat with fine detail, and a succession of overlapping and organized semicircles was clearly visible after carcass removal (Fig. 4.4E). Coccoid bacteria could also be distinguished at higher magnification, (Fig. 4.4F). This imprint corresponded to the external relief of the original scales of the fish, characterized by concentric lines (Fig. 4.4G). Energy-dispersive X-ray spectrometry (EDXS) confirmed the calcium phosphate composition of the scales, in contrast with the organic composition of the impression (see Supplementary Fig. S4.2).

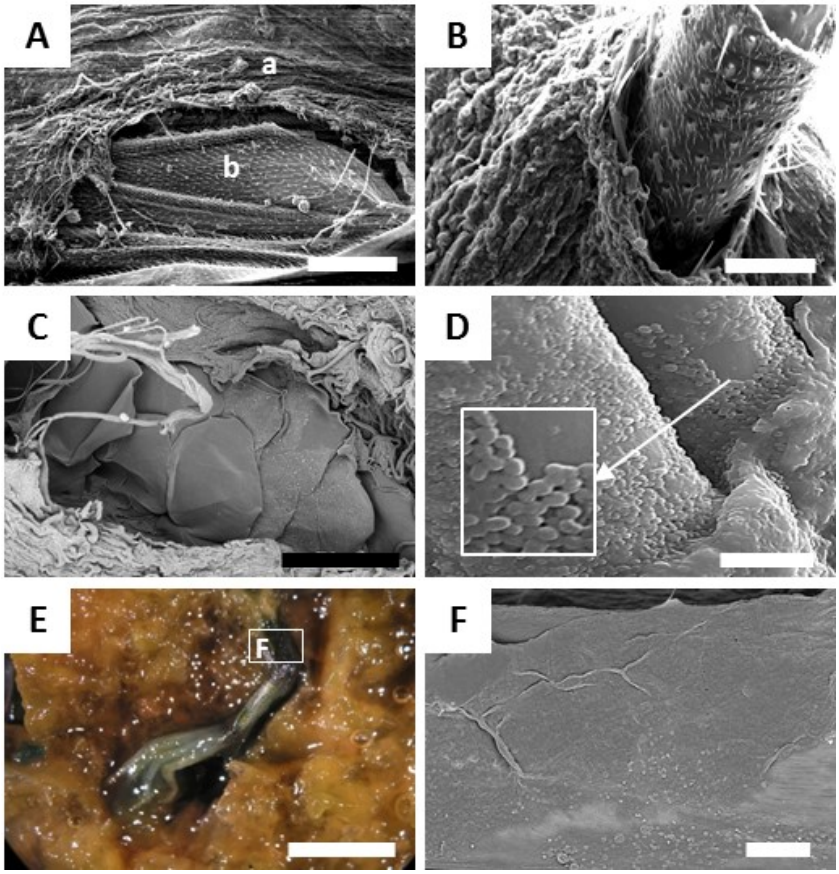


Fig. 4.2: Microbial mould of the external shape of carcasses. (**A**) SEM image of the fly wing (8 months): a, microbial coverage; b, wing. (**B)** Microbial sheath coating the fly femur. (**C**) Neon tetra fish tail, covered by a thick mat layer after 8 months. Fish scales are visible in the centre of the image. (**D**) Red carp fishbone after 15 months; the EPS matrix and coccoid bacteria are distinguishable. (**E**) Image under the binocular of frog foot and toes trapped within a mat, after 8 months. (**F**) SEM detail of the leg, showing the preserved surface of the skin. **A-D** and **F** are SEM images. (Scale bar: A, 200 µm; B, 50 µm; C, 500 µm; D, 10 µm; E, 5 mm; F, 150 µm).

Strikingly, the fish eyes remained clearly recognizable even after 24 months within the mat (Fig. 4.4H) and showed the lens and three layers (Fig. 4.4I) likely corresponding to the conjunctiva, cornea and iris. The observation of these areas at higher magnification revealed that each layer was actually a replica formed by EPS in which numerous short rod-shaped bacteria were embedded (Fig. 4.4J).

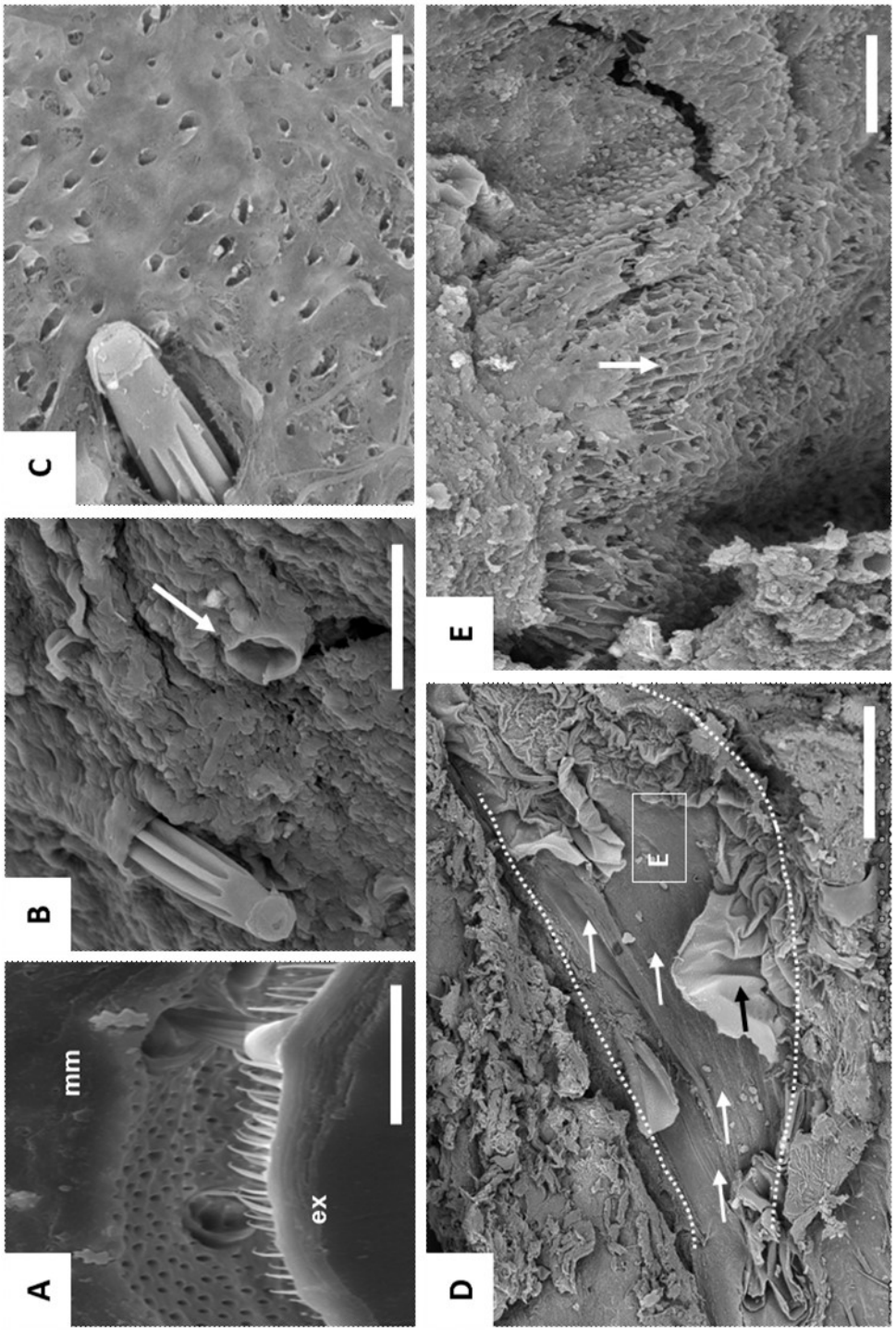


Fig. 4.3: SEM images of the finely detailed imprints on the mat. (A) Detail of the fly exocuticle setulae (ex) and their imprints on the microbial mat (mm). (B) Fly setae penetrating the microbial coverage; note the preservation of imprints even in the case of lost setae (arrow). (C) Setae trapped in a microbial mat and impressions of setulae. (D) Impression of the fly wing; white arrows point to the vein impressions. The black arrow indicates the original wing tissue trapped in a microbial layer and the dotted white line shows the outline of the wing. (E) Pattern of holes left by the wing hairs as an impression on the mat (arrow). (A, D and E) 8 months fly; (B-C) 5.5 years fly. (Scale bar: A-C, E, 20 µm; D, 500 µm).

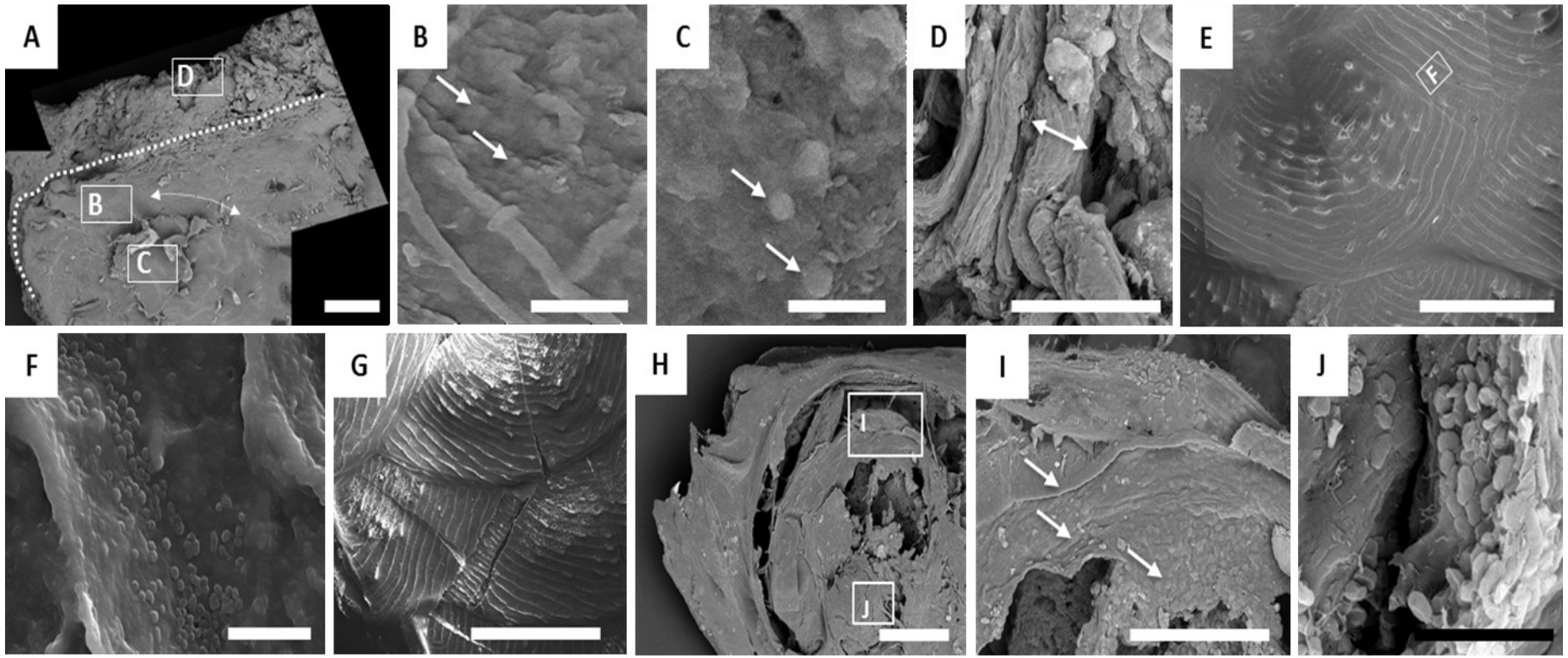


Fig. 4.4: SEM images of the impression of fish bodies on the microbial mat during the entombment process. (A) Mould of the head of a neon tetra fish after 24 months; three spots are marked and magnified in the following panels (B, C and D). Dotted line: imprint of the head; arrows: circular edge of the eye. (B) Impression of the eye with filamentous and rod-shaped cells (arrows) embedded within the EPS matrix. (C) Detail of the replica of the eye lens which was trapped in the mould after the removal of the head. Arrow points small coccoid cells (D) Regular microbial mat in an area outside the imprint, composed mainly of packages of Coleofasciculus filaments (see the package indicated by arrows). (E) Impression of the scales of a red carp after 15 months. (F) Detail of the impression of a fold in E. (G) Fish scales with an exceptional conservation of the original organization. (H) Head of neon tetra fish (24 months) where the eye structure seems to be still preserved (see panels I and J). (I) Detail of the superposition of microbial layers that replicate and replace the original eye tissues. Arrows: layers composed of an EPS matrix with embedded bacteria. (J) Replica of the eye lens replaced by microbes and EPS matrix. (Scale bar: A, E, G, H, 500 µm; B, C, F, J, 10 µm; D, 50 µm; I, 200 µm).

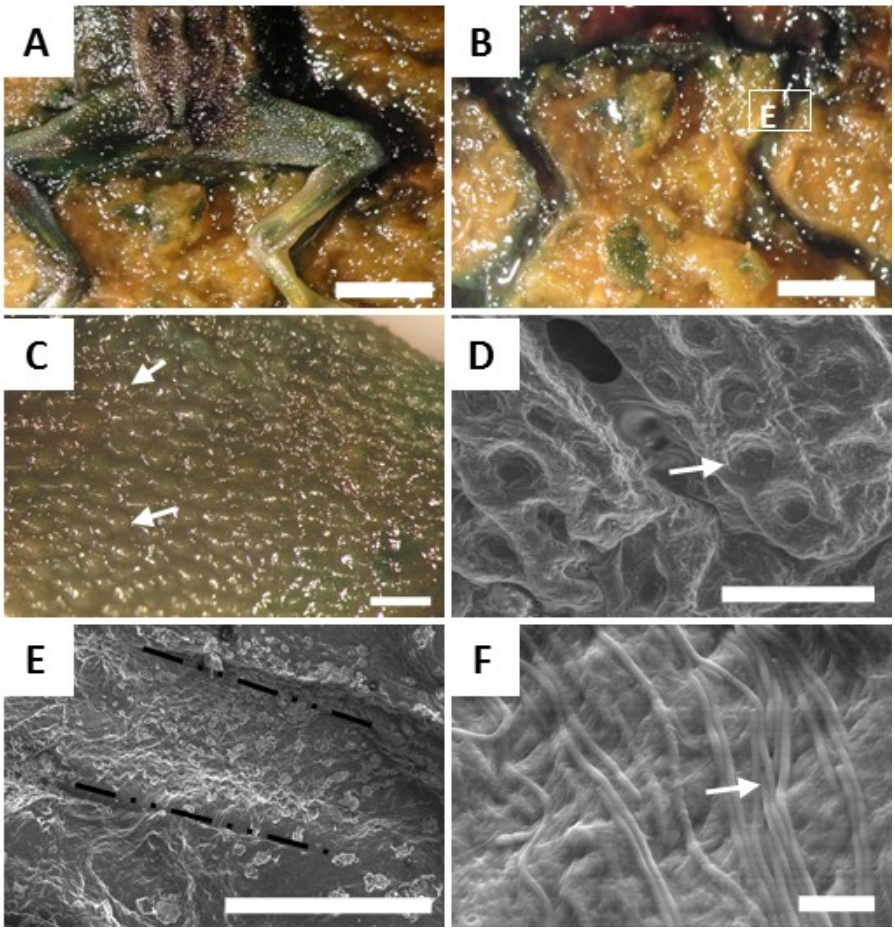


Fig. 4.5: Formation of the impression of frogs on the microbial mat. (**A**) Frog laying on the mat after 12 months once the upper layer of the sarcophagus was removed. (**B**) Hind limb impression. (**C**) Detail of the skin showing verrucae (arrows) after 8 months. (**D**) Environmental SEM image of the verrucae impressions (arrow) on the microbial mat. (**E**) SEM image of the microbial mould of a leg after 12 months. (**F**) Microbial filaments at the base of the imprint. (Scale bar: A, B, 2 mm; C-E, 500 µm; F, 20 µm).

Frogs covered by mats underwent a substantial reduction of body thickness after 12 months but the tegument remained intact, enabling the formation of an accurate impression of the whole carcass which was noticeable on the mat surface (Fig. 4.5A and B). The soft skin of frogs leaved a clear imprint in the mat and typical structures of the skin surface, such as the conspicuous aligned verrucae (Fig. 4.5C), were also visible as negative prints on the mat (Fig. 4.5D). SEM observation of the mat around the legs (Fig. 4.5E) showed visible cyanobacterial-like filaments embedded in an EPS matrix (Fig. 4.5F).

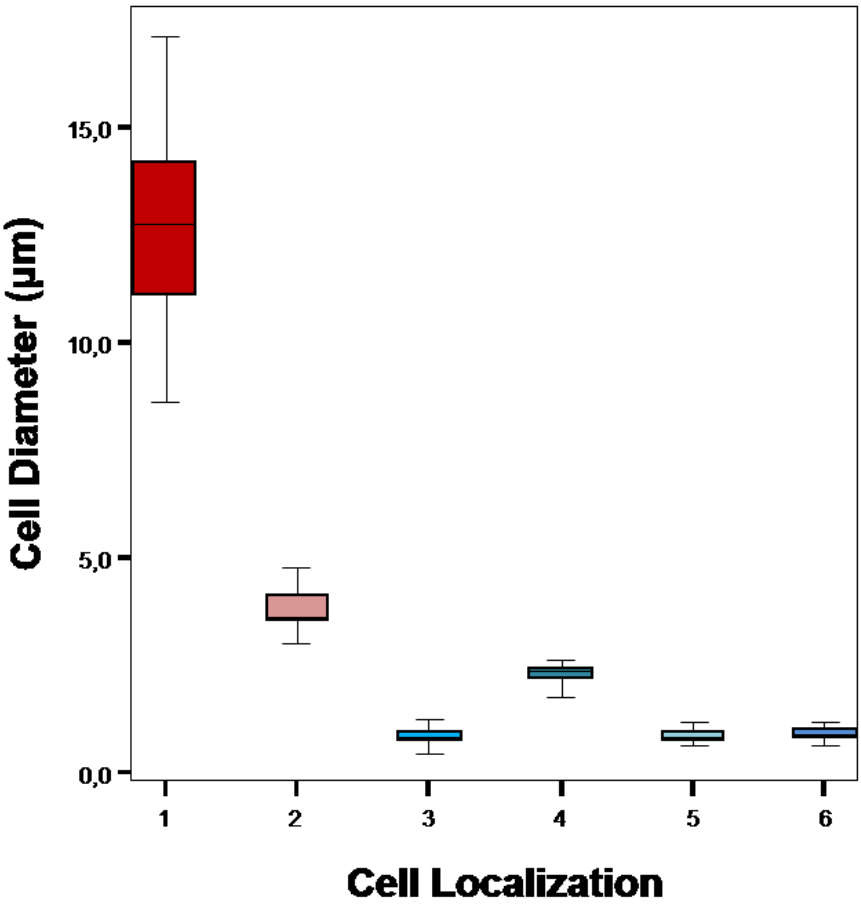


Fig. 4.6: Box-plot built with the measurements of cells present in SEM images of body impressions of fly wing (N=118), fish scales (N=270) and bones (N=130), and frog skin (N=74) and from the upper layers of the mat far from the animal bodies (N= 90). Localization: 1, Coleofasciculus thick packages (see Fig. 4D); 2, other cells in the upper layers of the mat; 3, cells over fish scales (see Fig. 4F); 4, filaments in the mould of the frog (Fig. 5F); 5, cells over fish bones (see Fig. 2D); 6, cells in fly impression (Fig. 3E). Upper and lower fences represent Q3 + 1.5 IQR (InterQuartil Range) and Q1 - 1.5 IQR respectively. Median is represented into the box by a black line. Marks out of the fences represent outliers.

A careful observation of impressions evidenced different sizes of microbial cells depending on their distance to the bodies. We measured the diameter of cells on the body impressions of the fly wings (N=118), the fish scales (N=270) and bones (N=130), frog skin (N=74), as well as cells from the upper layers of the mat not in direct contact with the bodies (N= 90). At the upper layer of the mat, the *Coleofasciculus* packages measured 12.77 ± 2.11 µm in thickness, whereas other smaller cells present in this layer had a diameter of 3.65 ± 0.79 µm. The size of cells composing the mould of the fly wing, scales and over fish bones ranged between 0.8 and 1 µm (Supplementary Table S4.1), although one smaller cell group (around 0.7 µm) was observed in the case of scales.

Filaments present in the mould of the frog measured 2.27 ± 0.21 µm in thickness, being also smaller than cells lying on the top (Supplementary Table S4.1). A box plot (see Fig. 4.6) shows the existence of different cell size populations consistent with the different localizations.

Conservation of carcasses and inner soft parts

Animal bodies were degraded gradually within the microbial mats but the decay rate was much slower than that observed in control corpses maintained in tanks without microbial mats, in agreement with previous experiments using fish bodies (Iniesto et al. 2013). Flies, which have a hard cuticle, retained their exoskeleton practically intact even after 5.5 years within the mat, and even complex organs, such as the fly ommatidia, still conserved their original organization after this long time (Fig. 4.7A), and some inner soft-tissues were yet present (Fig. 4.7B). Although internal structures were in general difficult to identify, several inner soft-tissues, most likely the ovary and the digestive tract of the flies, were visible in resin-embedded sections (Fig. 4.7C). By contrast, flies in control experiments without microbial mats were completely degraded after 6 months. Preservation of inner tissues was also observed in the fish bodies covered by mats. After 8 months, magnetic resonance imaging (MRI) revealed the presence of inner organs such as the swim bladder and muscle bundles (Fig. 4.7D). The 24-months fish kept the articulated backbone (Fig. 4.7E) and its swim bladder (Fig. 4.7F) as observed by SEM in resin-embedded sections. In frogs, the articulation of the skeleton remained unbroken (Fig. 4.7G) and the skin maintained its integrity. Even delicate structures such as the membrane between fingers (see Supplementary Fig. S4.3A and B) and the small protuberances that composed the surface of the skin (Supplementary Fig. S4.3C and D) were preserved after 12 months. In addition, the section of the frog femur showed organic tissue inside, which may correspond to rests of the bone marrow also preserved after 12 months (Fig. 4.7H and 7I). No microorganisms appear to be present in this section. Prokaryotic cells seemed to be also recurrently absent inside fish and flies.

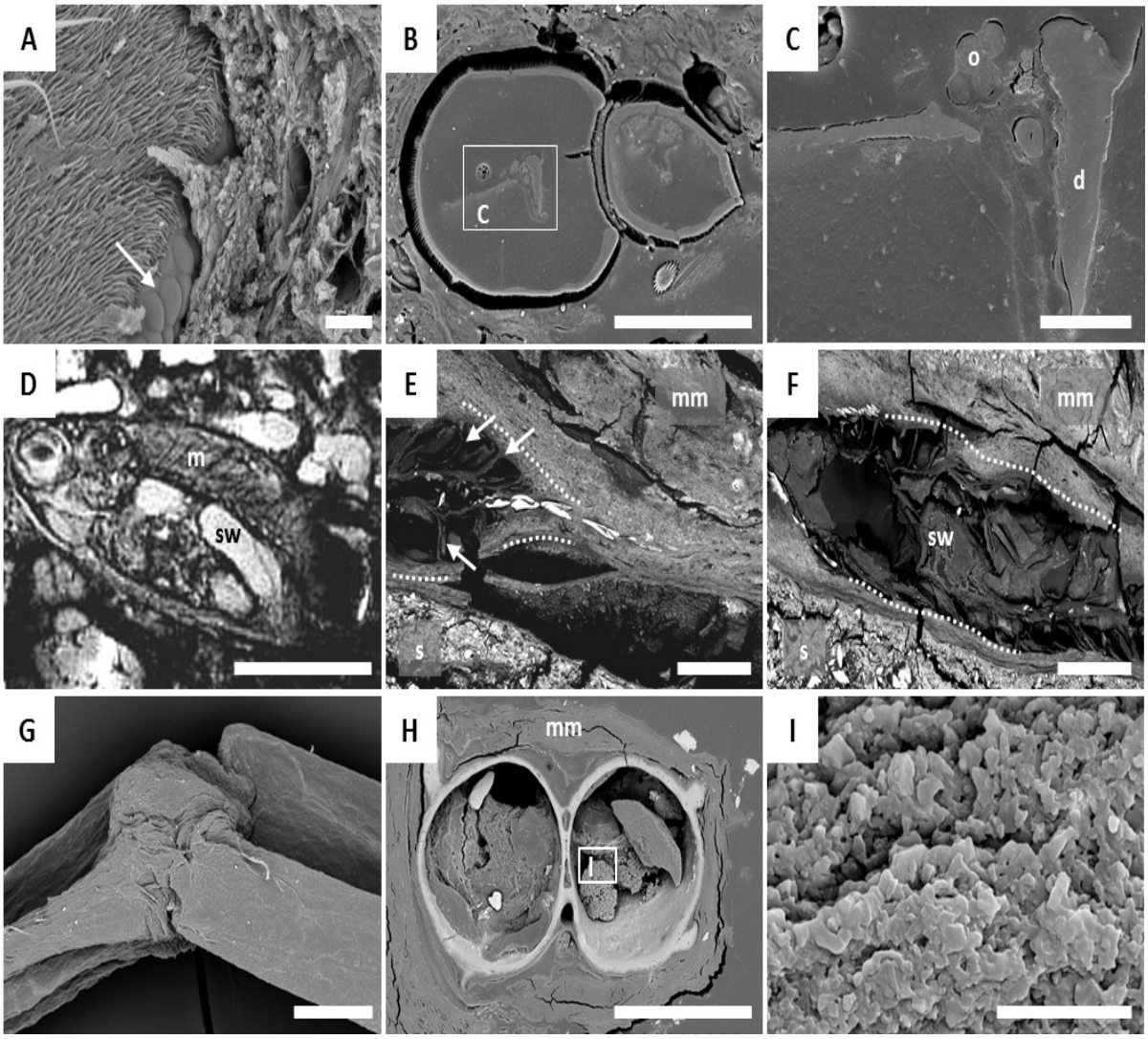


Fig. 4.7: Preservation of animal tissues after prolonged periods of residence in the microbial mats. (**A**) Ommatidia (arrow) of a fly after 5.5 years. (**B**) Epoxy-embedded section of a fly (abdomen and thorax) after 5.5 years. Light grey structures inside it correspond to preserved inner soft tissues. (**C**) Detail of soft tissues preserved in the abdomen. According to the size, organization and position, these organs are most likely the ovary and digestive tract. (**D**) MRI of an exceptionally preserved fish (Paracheirodon innesi) after 8 months. (**E**) Tail of a 24-months preserved fish. Arrows: supporting tissues around the vertebral column; (**F**) Section of a fish completely embedded in the microbial mat (24 months). Dotted lines in E and F indicate the external shape of the fish. (**G**) Fully articulated leg of a frog after 12 months. (**H**) Epoxy-embedded section of the tibia and fibula. (**I**) Detail of the preserved bone marrow in H where microorganisms appear to be absent. Images acquired by SEM (except **D**). Abbreviations: d, digestive tract; m, muscle boundless; mm, microbial mat; o, ovary; s, sediment; sw, swim bladder (Scale bar: A, C, 20 µm; B, 100 µm; D, 5 mm; E-H, 500 µm; I, 10 µm).

DISCUSSION

Our experiments show that the formation of a sarcophagus around carcasses by the microbial mat community has important consequences regarding the initial phases of the taphonomic process. The presence of microbial mats reduces the time of the entombment of carcasses to a few days (less than 20 days in our experiments), forming a sarcophagus composed of microbial cells embedded in an EPS matrix that generates a physical and chemical environment distinct from ambient conditions. In addition, the sarcophagus provides the template for the formation of moulds and imprints, and it promotes the replica of soft organic structures. Our experiments illustrate three important facts: the coherence of the mat upper layer, necessary for the imprint formation once the body has been covered; the presence and distribution of small coccoid cells around the carcasses; and, finally, the conservation of the inner soft tissues, even for long periods of residence of the bodies within the mats.

As explained above, the coat formed on the animal bodies by the EPS matrix with embedded microbial cells is able to copy the shape and details of positive structures such as the fly wing venation, the fish scales or the verrucae of the frog skin and also very fine details such as the 3-4 µm impressions left by the fly setulae and wing hairiness. The implication of microbial mats in the formation of high-fidelity impressions is widely accepted (e.g. Gehling 1999, Narbonne 2005 and (Steiner y Reitner 2001), and Darroch et al. (2012) highlighted experimentally the formation of “death masks” by mats, developing the model proposed by Gehling (1999). In our experiments, imprints were observed both on the top and the bottom of the carcasses, in contrast with the prior assumption of a differentiate preservation of masks depending on the face.

Small coccoid or slightly elongated prokaryotic cells less than 1 µm in size (usually embedded within the dense EPS matrix) appeared either coating structures (such as the fish bones) or forming part of the impressions. Small filamentous prokaryotic cells, with an average diameter of 2 µm, were also present in the mould of frogs. The size of prokaryotic cells seemed to be different depending on the proximity to the carcasses: large cells (such as *Coleofasciculus* thick packages) were located externally, while small cells were in close contact with the bodies. However, further studies will be needed to clarify the functional role of the different prokaryotic species along the microbial succession related with early diagenetic processes. The occurrence of small spherical structures in the fossil record has been frequently interpreted as coccoid prokaryotic cells. This is the case of the dinosaur *Pelecanimimus* from the Early Cretaceous Konservat-Lagerstätten of Las Hoyas (Spain), which was covered by a purported phosphatized microbial mat containing layers of coccoid cells embedded in a film (Briggs et al. 1997. It was shown that the microbial mat replicated the structure of the muscles and skin of this dinosaur. In addition, negative impressions of coccoid and rod shaped bacteria were observed on the bone surface of teleostean fish from the same deposit (Gupta et al. 2008). In the Miocene locality of Libros (Spain), articulated skeletons of frog and salamander specimens were discovered enclosed in a thin carbonaceous bacterial

biofilm with fossilized coccoid-shaped or slightly elongated bacterial cells (McNamara et al. 2006). The horseshoe crab fossils from the Upper Jurassic Nuspligen site (Germany) also showed mineralized biofilms with coccoid and spiral forms in association with muscles (Briggs et al. 2005). In the present study, we observed bacteria with the same shape and size in the replicate of the eye of the fish, consistently with what has been observed in fossils. In fact, Martill (1987) and Gupta et al. (2008) described carbonaceous laminae formed by a microbial film replacing vertebrate eye soft tissues in marine and alkaline Mesozoic waters. Moreover, melanosomes, which are organelles present in most animal eyes, have been recognized in a Lower Cretaceous bird (Vinther et al. 2008) and in an Early Eocene fish (Lindgren et al. 2012). However, the distinction between melanosomes and microbes is controversial (Moyer et al. 2014). Taking into account the criteria proposed by Moyer et al. (2012) to differentiate them, we consider that the structures observed in the present work were most likely prokaryotic cells according to their distribution and density, which would be congruent with the progressive colonization of the tissue by microbes.

The organic sarcophagus not only outlines the detailed three-dimensional shape of the animals, but it also protects them and might be involved in subsequent (bio)mineralization. Even the interior of the animal bodies is protected from substantial decay, as evidenced by the preservation of the ovary and digestive tract in flies or the swim bladder in fish after many months (up to 5.5 years in the case of flies). The preservation mediated by microbes of this kind of inner tissues (i.e., digestive tract) has already been highlighted experimentally by Butler et al. (2009) and Butler et al. (2015) but the preservability of sexual organs had never been evidenced before. Previous experiments conducted by Briggs and Kear (Briggs and Kear 1993, 1994) using heterotrophic microbes explained this delayed decay over only two months by the development of anoxia. In the case of mats, formed by microbial communities much more complex than those used by consistence, the implication of anoxia in tissue preservation could be important in the earlier phases of coverage (several days), but not all along the process because tissues inside the fish, which were preserved over a year, become oxic after three months and forward, as shown by Iniesto et al. (2015b). Moreover, in the present study, frog bones still have an intact medulla after 12 months. This kind of tissue is and seldom preserved and, therefore, very rare in fossils. Noticeable exceptions are the observation of medulla in *Tyrannosaurus rex* (Schweitzer et al. 2005) and in the anuran *Pelophylax pueyoi* (McNamara et al. 2006) (formerly named *Rana pueyoi*) being this last one likely related with mats preservation.

It has been suggested that the sealing effect of the microbial mat can be even more important because the whole EPS organic matrix may work as a chelator for cations and then drive mineral crystal nucleation (Costerton et al. 1995, Decho and Kawaguchi 2003, Kühl et al. 2003). In a previous work, we described a Ca-rich thin veil on fish specimens after only 1 month of incubation within the mats (Iniesto et al. 2013). Hence, the microbial activity may generate specific microenvironments favouring mineral precipitation and further protection against degradation (e.g., Li et al. [2013]). Although the occurrence of biomineralization in taphonomic experiments has been linked

frequently to acid conditions (Briggs and Kear 1993, Wilby et al. 1996), this assumption may not be correct in the case of microbial mats. In fact, preservation can be initiated through other mineral phases, such as carbonates (McNamara et al. 2009), calcium phosphate (Benzerara et al. 2004) or Mg-rich silicates (Souza-Egipsy et al. 2005), favoured by the basic and oxic conditions already described during carcass preservation in mats (Iniesto et al. 2015a). For example, the occurrence of a talc-like mineral phase in intimate contact with the upper face of a fish embedded in a mat for 5 years has been described (Iniesto et al. 2015b). Precipitation of such mineral phases at the interface between the carcass and the mat most likely leads to a longer preservation of the impression by increasing the probability of fossilization.

Our results confirm the relevance of mats in two major taphonomic processes: replication and moulding. Their occurrence depends on the ecological factors affecting mat growth. It is noteworthy that, according to their palaeoecological description (Buscalioni and Fregenal-Martínez 2010, Mcnamara et al. 2010), several relevant Konservat Lagerstätten (e.g. Libros or Las Hoyas) presented at the time when their fossils were formed the environmental conditions conducive to the development of microbial mats (i.e., shallow lakes, ponds, water conditions, seasonality, high pH, etc.). Fossils from these sites show features similar to those that we observed in our experiments. The cast of external features of *Pelecanimimus* (Briggs et al. 1997) is an unequivocal example of the moulding ability of mats herein described, which also applies to the carbonate-based anuran outlines studied by McNamara et al. (2009) and Mcnamara et al. (2010). Actually, the formation of imprints in both faces of the carcasses and the preservation of external morphologies are the result of the inherent features of the microbial sarcophagus (i.e., coherence, full coverage of the body, microenvironmental conditions and high-fidelity impressions). The present work identifies processes of preservation which may explain many characteristics of the fossil record collected in sites with indications of contemporary microbial mat growth. According to experimental evidence and the study of the fossil record, the assumption that microbial mats are crucial for these pathways of microbially-mediated preservation is plausible. In addition, our results enlarge the significance of microbial mats in replication and moulding, following the model for the formation of fossils from Ediacara proposed by Gehling (1999), and confirmed experimentally by Darroch et al. (2012).

A quick burial and a delayed decay are two conditions that are considered to be necessary for exceptional preservation (Gaines et al. 2012). Traditionally, rapid burial was most often supposed to occur by sudden entrapment within sediments. However, microbial mats offer an interesting alternative as that microbial community can cover carcasses rapidly by the colonization of the body surface. In addition, mats also fulfil the second condition as they clearly slow down the decay of the bodies, which are protected and preserved inside the mats through the formation of a sarcophagus. Our experiments show a rapid growth of mats around the bodies and a notable delay of their decomposition for the three types of organisms used in our study, both vertebrates and invertebrates, suggesting that it can be a general phenomenon. Moreover, microbes were shown to be involved in the replication of original structures. The direct contact of

the prokaryotic cells (and their secreted EPS) with the carcasses creates accurate impressions of their surfaces. This intimate relationship between a macroscopic organism and microbial mats is most likely capital for its preservation (Supplementary Fig. S4.4). Despite the progress in experimental taphonomy, the mineralization of replicas and impressions still remains poorly understood. Longer experiments will be needed to further characterize the relationships existing between the metabolic activity of the microbial community and early diagenesis.

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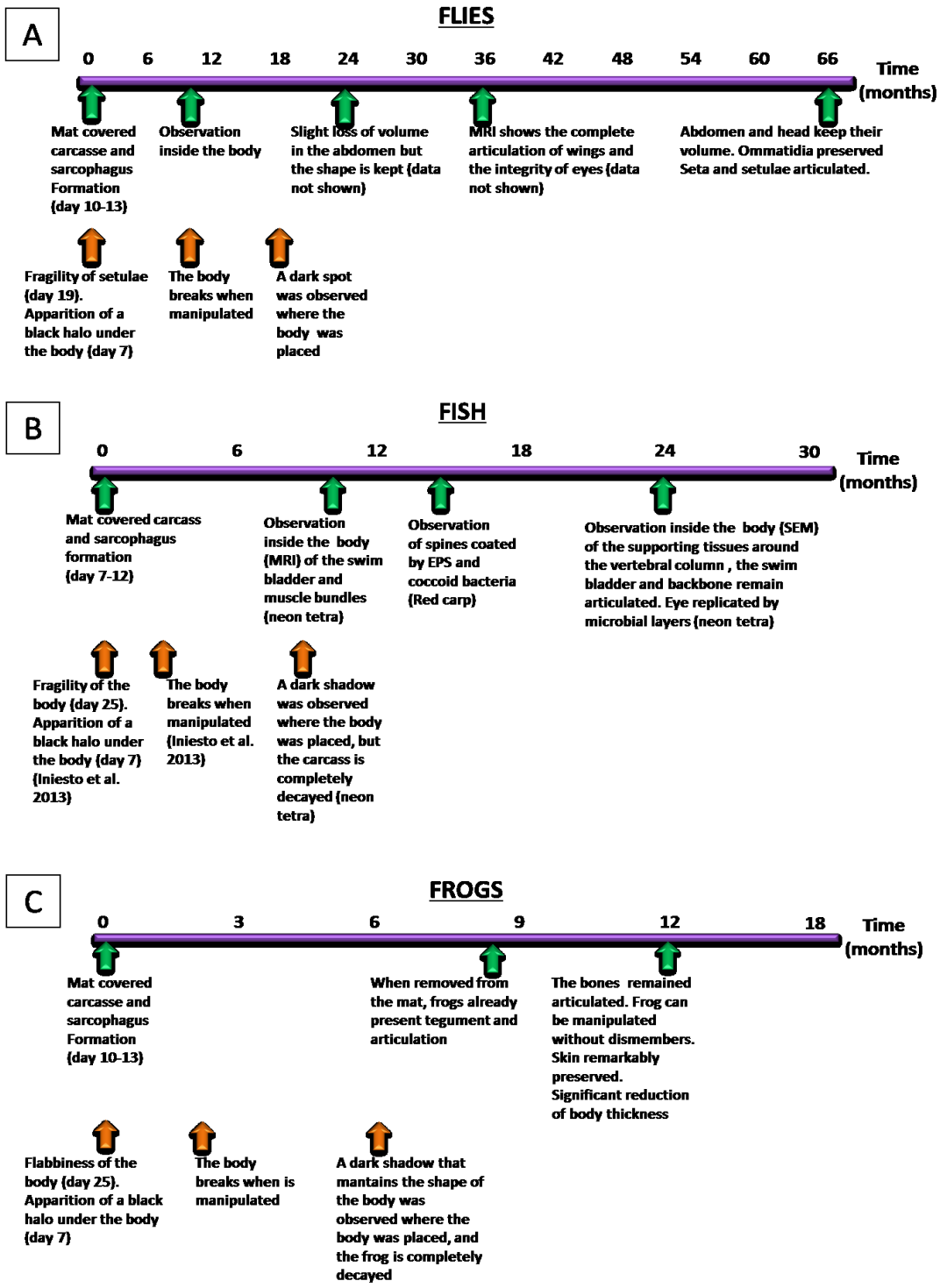
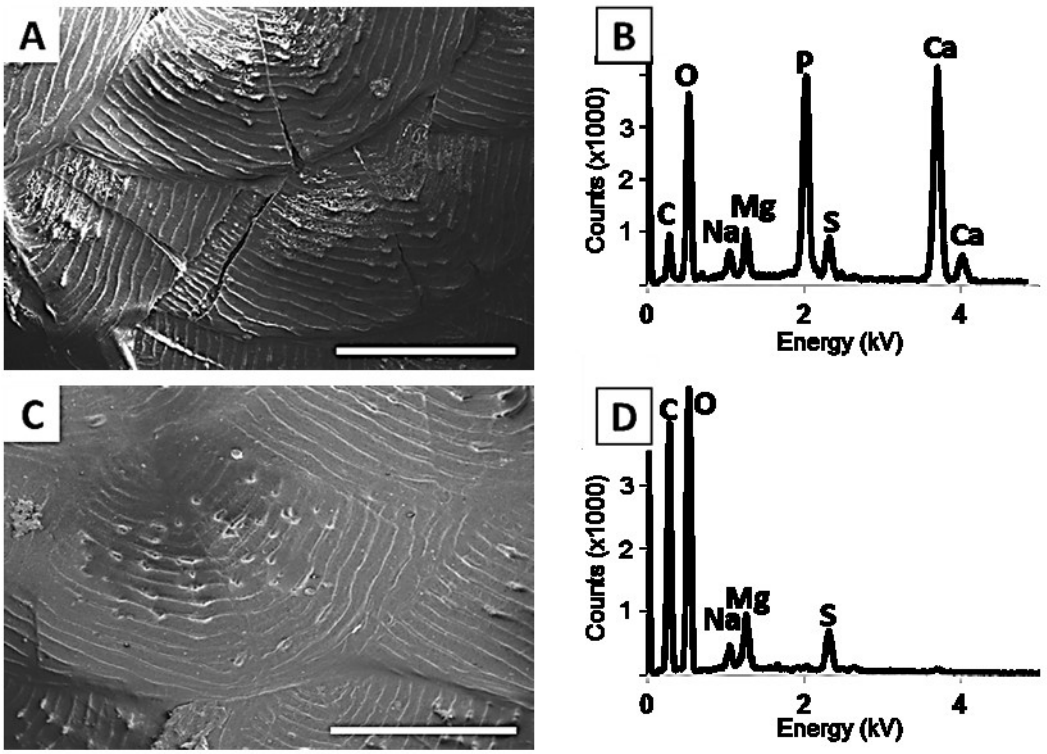
SUPPLEMENTARY FIG S4.1



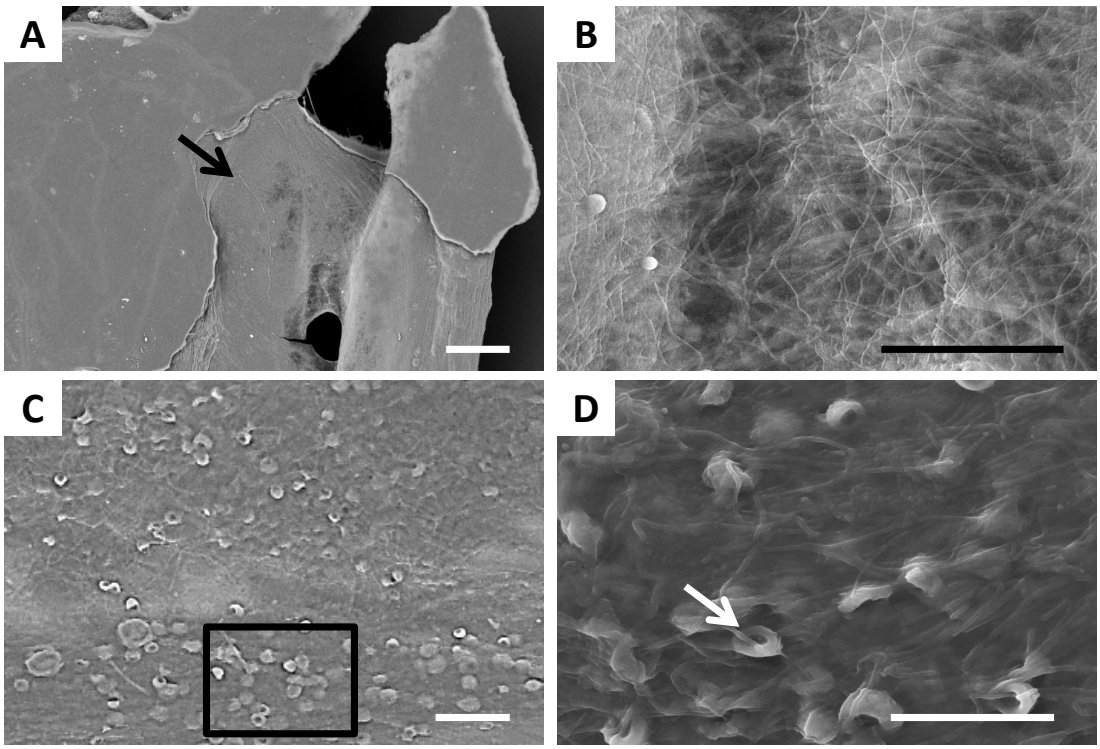
Supplementary Fig. S4.1: The diagram summarizes the sampling and several relevant events monitored during decay of flies (**A**), fish (**B**) and frogs (**C**) in mats (green) and controls over sediment (orange). Each arrow represents at least two bodies analysed in order to describe the decay.

SUPPLEMENTARY FIG S4.2



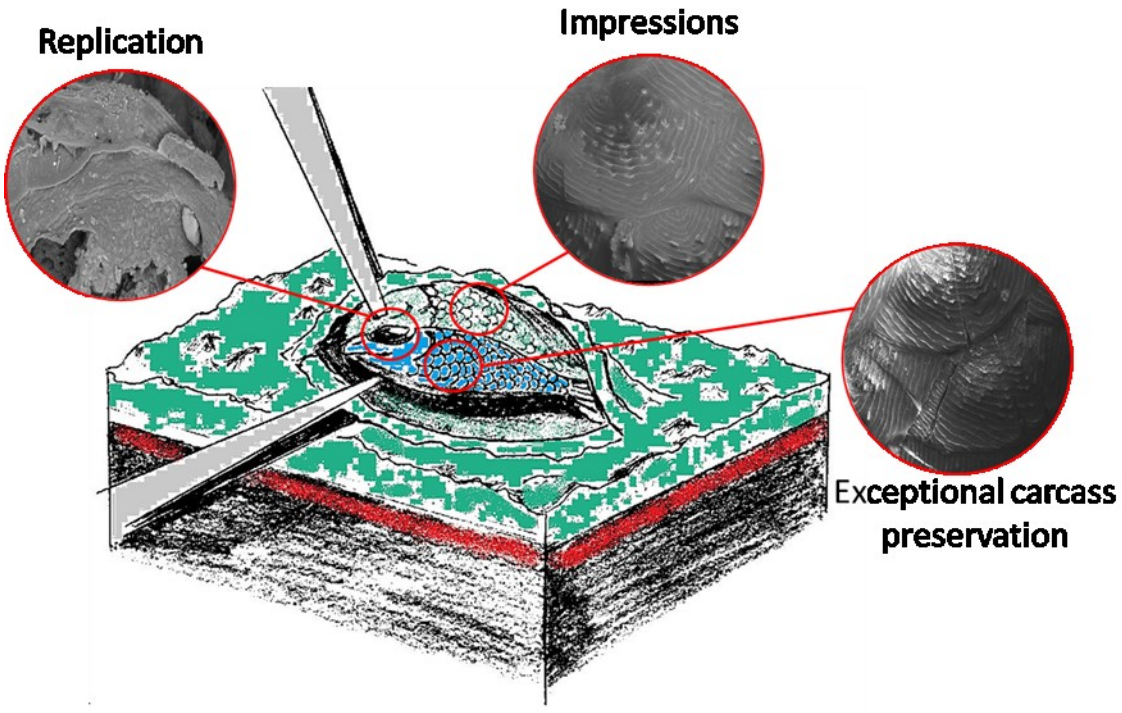
Supplementary Fig. S4.2: Energy-dispersive X-ray spectrometry (EDXS) of scales of neon tetra fish and the impression generated in the microbial mat. (**A**) Scales of neon tetra fish and the corresponding EDXS spectrum (**B**). (**C**) Impression generated in the microbial mat and its EDXS spectrum (**D**). EDXS confirmed the mineral composition of the scales, made mainly of calcium phosphate, in contrast with the organic composition of this impression. Scale bars: 500 µm.

SUPPLEMENTARY FIG S4.3



Supplementary Fig. S4.3: Integrity of frog carcasses while covered by mats after 12 months. (**A**) Interdigital webbing of the frog. The surface is partially covered by the mat, but the integrity is remarkable in those zones in which the membrane is exposed (arrow). (**B**) Skin of the frog at higher magnification, where skin-deep blood vessels are evident. (**C**) Surface of the leg of frog with skin still covering the appendix. (**D**) Detail of the surface showed in **C**. A large number of microbial cells are embedded in a matrix that covers the tegument, and small protuberances of the skin (arrow) rise to the surface despite the microbial film after 12 months. Scale bars represent 200 µm in **A**; 50 µm in **B**; 70 µm in **C**; 30 µm in **D.**

SUPPLEMENTARY FIG S4.4



Supplementary Fig. S4.4: Schematic diagram of the preservation of carcasses (a fish in the example) into a microbial mat. After the coverage of the mat, the intimate contact of the upper layers of the mat and the surface of the body results in the formation of fine impressions and replicas. In addition, the sarcophagus leads to an exceptional delay of decay and the preservation of tissues, even inner soft-tissues.

SUPPLEMENTARY TABLE S4.1

Supplementary Table S4.1: Data of the measuring of the different cells observed at SEM.

	N	Meaning Value	SD
Cells in Fly Mould	118	0.828	0.133
Cells over scales	270	0.802	0.165
Small Cells over Fish Scales (<0.85)	159	0.701	0.107
Medium Cells over Fish Scales (>0.85)	111	0.959	0.088
Cells over Fish Bones	130	0.852	0.134
Filaments in Frog Mould	74	2.267	0.206
Filaments from the upper layers	65	12.77	2.11
Cells from the upper layers	25	3.65	0.79

CAPÍTULO 5

THE IMPACT OF MICROBIAL MATS AND THEIR MICROENVIRONMENTAL CONDITIONS IN EARLY DECAY OF FISH

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ABSTRACT

This study provides experimental evidence confirming the significance of microbial mat presence in controlling the spatial and temporal development of chemical microenvironments which become established inside and outside decaying fish carcasses. Dissolved oxygen (DO) and pH profiles were monitored over 1,000 days with microelectrodes positioned inside and adjacent to carcasses. In the vicinity of fish, the DO varied from an oxygenated green upper layer to an anoxic bottom stratum and the pH was alkaline. Inside the fish the DO and the pH were decoupled during the first week, in that anoxia remained constant and the pH decreased and became acidic. Once covered by the mat, the fish carcass turned oxic and pH returned to alkaline levels. This novel second phase occurred from day 90 to three years and would likely remain after this time. Based on the fish soft tissue quality at the end of the experiment, we discuss whether oxic-alkaline microenvironmental conditions could promote long-term mineralization.

Keywords: bacterial sealing, delayed decay, experimental taphonomy, microsensors, preservation

RESUMEN

Este estudio confirma de forma experimental la implicación de los tapetes microbianos en el control espacial y temporal del microambiente químico que se genera alrededor y en el interior de cadáveres de peces en descomposición. El oxígeno disuelto (DO) y el pH fueron analizados mediante microelectrodos, durante un periodo que llegó a los 1000 días, tanto en el interior de los cuerpos como en su entorno inmediato. Cerca de los restos el DO fue cambiando desde valores altos en las capas superiores del tapete a condiciones de anoxia en las capas más profundas, con un pH que se mantuvo predominantemente alcalino. En el interior de los cadáveres se observó que el pH y el DO variaban de forma desacoplada en la primera semana, con valores constantes y bajos de DO y un pH que descendió hasta la acidez. Una vez cubiertas por el tapete, las carcasas de peces volvieron a estar óxicas y alcalinas. Esta segunda etapa se extiende desde los 90 días hasta los 3 años, por lo que podría continuar en periodos aún más largos. De acuerdo con la integridad que presentan los tejidos blandos de los peces a la finalización del experimento, se discute si las condiciones microambientales óxicas y alcalinas podrían promover la mineralización a largo plazo.

Palabras clave: microelectrodos, preservación, retraso en la descomposición, sellado bacteriano, tafonomía experimental

INTRODUCTION

The fossilization of organisms involves changes which occur before and/or after burial (biostratinomy and diagenesis, respectively) (López and Trujols 1994). Although these changes almost invariably result in loss of information, certain physical and chemical conditions may enhance soft tissue preservation, allowing in some cases the retention of details in fossilized soft tissue at the cellular or even molecular levels (Asara et al., 2007; Schweitzer et al., 2007, 2009; McNamara et al., 2009). A key factor routinely involved in the genesis of such exceptional deposits (Konservat Lagerstätten) is what can broadly be termed "bacterial sealing" (Seilacher et al., 1985). It has been long recognized that what can be termed Microbially Induced Sedimentary Structures (MISS) (Noffke, 2009; Noffke and Awramik, 2013) enhance the likelihood of soft tissue preservation. Soft-bodied organisms and soft tissues are frequently associated with evidence for the former presence of biofilms (Gall et al., 1985; Gall, 1990; Seilacher, 1990; Behrensmeyer, 2001; Briggs, 2003; O'Brien et al., 2008; McNamara et al., 2009; Buscalioni and Fregenal-Martínez, 2010; Pawlowska et al., 2012). Other investigations have used experimental procedures to elucidate the relation between aspects of exceptional preservation and bacterial activity. Sagemann et al. (1999) and Grimes et al. (2001) attempted to reproduce the biogeochemical processes involved in soft tissue mineralization, and Peterson et al. (2010), Daniel and Chin (2012), Iniesto et al. (2013) and Guerrero et al. (in press) all assessed how the composition and morphology of biofilms were related to tissue preservation and, critically, retention of the integrity of carcasses.

In this paper we describe an experiment designed to investigate the role of complex biofilms –i.e., microbial mats– in soft tissue fossilization. Unlike other experiments, in which the experimental vessel was inoculated only with some heterotrophic bacterial populations and monitoring comprised short time periods, this investigation analyses the processes which occurred inside a microbial ecosystem as a whole over an extended time period. Microbial mats are complex benthic communities organized as millimetric multi-layered microecosystems frequently dominated by cyanobacteria. These communities have a distinct vertical structure (Cohen, 1989) controlled by the extent of light penetration. Microbial mats are responsible for the biolaminated facies produced in certain lacustrine environments such as carbonate wetlands or lagoons (Fregenal-Martínez and Melendez, 2000; Noffke et al., 2013). Notably, this organization of the mat creates physical and chemical gradients as the result of the activities of the living microorganisms (Wierzchos et al., 1996; Hubas et al., 2011). The various layers of the microbial mat are in constant growth. Typically, cyanobacteria, which are oxygenic photosynthetic organisms, occupy the upper layer, while several phototrophic anoxygenic bacteria reside in an intermediate layer above yet lower layers comprised of other anaerobic bacteria, such as the sulphate-reducing species. Sediment particles, including organic remains and minerals precipitated in situ become trapped within the growing mat (Krumbein, 1979). In a previous experiment in which fish were covered and buried in microbial mats, Iniesto et al. (2013) demonstrated that the inner tissues

remained almost unaltered over more than two years (27 months). The key aspects of the interaction between the carcasses and the microbial mats consist of (1) replication of the body surface (Darroch et al., 2012); (2) a decrease in the decay rate, and (3) mineral bioprecipitation within the mat (Briggs, 2003; Kühl et al., 2003; Decho and Kawaguchi, 2003; Dupraz and Visscher, 2005; Ludwig et al., 2005; Iniesto et al., 2013). All of these processes can be initiated within a few days (Sagemann et al., 1999; Iniesto et al., 2013).

This paper concentrates on the significance of the microbial mats' local chemical conditions during the decay process of fish carcasses: it assesses whether these changes in environmental conditions promote preservation and enhance the likelihood of mineralization.

MATERIAL AND METHODS

Sampling and Microbial Mat Growth

Samples of microbial mats were collected from the Salada de Chiprana (Zaragoza, NE Spain), the only hypersaline and permanent inland shallow lake in Spain. It occurs in the Bajo Aragón semi-arid region and is part of the catchment area of the Ebro River. The Salada de Chiprana is fed by groundwater discharge which supplies abundant magnesium sulphate and sodium chloride. The lake has no outlet, as a result of which its salinity is very high, varying between 30 to 70%. Fluctuations in salinity are due to the changes in the volume of surface run-off which supplies the lake with abundant nutrients. The combination of this feature together with a high pH (values above 9) results in microbial mats being the prevalent communities covering an extension from the shore to a depth of 1.5 m (Guerrero et al., 1991). In their study of the physicochemical and biological features of the lake, Vidondo et al. (1993) recognized the lagoon as unique in Western Europe because of the growth of these dense microbial mats. The Salada de Chiprana mats are very compact due to dominance of the filamentous cyanobacterium Coleofasciculus chthonoplastes (Thuret ex Gomont) M. Siegesmund, J.R. Johansen and T. Friedl, (formerly known as Microcoleus chthonoplastes Thuret ex Gomont). The taxa comprising the upper mat layer are Chloroflexus, Coleofasciculus and Pseudoanabaena-like. The underlying darker layer is made up of dead cyanobacteria, frustules of diatoms and anoxic microbial populations (Jonker et al., 2003). More recently, Ludwig et al. (2005) observed how calcification of the mat occurred and revealed the importance of photosynthesis and sulphate reduction as agents driving the mineralization process.

Following collection in the field, microbial mats were grown in the lab under controlled conditions according to the protocols described by Iniesto et al. (2013). One well developed microbial mat was grown in a glass tank illuminated by a 50 W halogen lamp (Osram Decostar 46870WFL). Incident illumination approximated an intensity of 300 ± 1 µmols.seg-1.m-2, with a 12/12 hour dark/light regime to simulate the natural environmental conditions. A separate tank containing lake sediment was used as a control to monitor how decay progressed in the absence of a microbial mat. This tank was kept in the dark in order to prevent growth of photosynthetic communities. Initial physical conditions of the tank with mat were 27 ºC ± 0.5, a pH of 9.6 ± 0.5 and a conductivity of 57.1 mS·cm-1 ± 2.3. The corresponding values of the water column in the control tank were 26 ºC ± 0.5, 7.6 ± 0.2 and 58.2 mS·cm-1 ± 1.9. The initial differences in pH are the result of the biological activity within the photosynthetic mat. Sterilized deionized water was added periodically to the tanks to compensate for evaporation in order to avoid strong variations in both water level and salinity over the course of the experiment. In order to prevent the disruption caused by the water addition, tanks were filled 24 h prior to data collection thus allowing water column conditions to stabilize. No mixing system was employed and the water columns remained static.

Eight individuals of the small zebra fish Danio rerio were used in the experiments. They were euthanized immediately before the start of the experiment following the standard animal care protocol used by the Universidad Autónoma de Madrid. Four fish were laid out on the mat and four on the sediment surface (the controls), leaving sufficient space between fish to avoid any interaction (e.g. the halo of cyanobacterial growth stimulation described in Iniesto et al. [2013]). Results reported previously (Iniesto et al., 2013) indicate that the mat-carcass system undergoes major and rapid changes during the first days. Subsequently, in the following days, the decay process stabilizes as soon as the carcasses are covered by the mat. The current assay reproduces the exact same experimental conditions, thus, the same relationship between the extent of decay and changes over time in the microenvironmental conditions was inferred. Therefore, measurements were taken after 2, 7, 90 and 1,000 days with respect to the microbial mat tank and after 2, 7 and 90 days in the case of the control tank. At day 1,000, decay of the control fish was complete (Iniesto et al., 2013) and therefore no analyses were possible. Only one fish was measured at each experimental time. Measurements were collected twice in contiguous points to serve as pseudo-replicas. Data was also taken before each fish carcass was placed to define the initial conditions.

Profile Determination and Data Treatment

Dissolved oxygen (DO) and pH profiles were measured using the MiniProfiler MP4 microelectrode system (Unisense Corp., Denmark). For each fish, two zones were analyzed: (1) adjacent to the fish, corresponding to the area that turned dark green following the deposition of the fish (Fig. 5.1: point NF), and (2) in the centre of the fish's body continuing through the fish into the sediment below the microbial mat on which the fish was placed (Fig. 5.1: point IF). The dissolved oxygen and pH values were also measured in an area distant to the fish bodies at the beginning of the experiment, representing an additional control on the substratum conditions unaffected by the presence of the carcasses.

Microelectrodes were calibrated at the beginning of each analysis. For dissolved oxygen the calibration equation of Jørgensen and Revsbech (1985) was used. Saturation concentration values were obtained by measuring the dissolved oxygen concentration in a glass of water after bubbling air through it for 10 minutes. The pH electrode was calibrated using a three-point calibration using Crison(R) buffers of pH 4, 7, and 10. Profile depths were determined using methods appropriate to each experimental set up. Depths were easily determined as the walls of the glass tank were transparent. The regularity of the sediment surface (see also the mat and control provisions used by Iniesto et al., 2013) allowed each profile to be aligned. In all cases measurement showed that oxygen and pH conditions reached stable readings a few millimeters into the sediment;we have assumed that variables remained stable below this depth. For each profile, data of each pseudo-replicate were taken every 0.5 mm. Error bars in plots show the standard variation of pseudo-replicates. To guarantee precision and to minimize analytical method bias, means were derived from 5 measured values at the same depth

holding the microsensor immobile. The experimental setting determined the number of points to be checked throughout of the profile, ranging from 32 (microsensor through the sediment only of the control tank) to 42 (through the fish, the microbial mat and the sediment of the mat tank). Data span 14 mm of each profile was selected to compare equivalent levels in each tanks, taking the surface of the mat or the sediment (in the case of controls) as reference. Plots do not present lower depth profiles where the electrode detected no further variation.

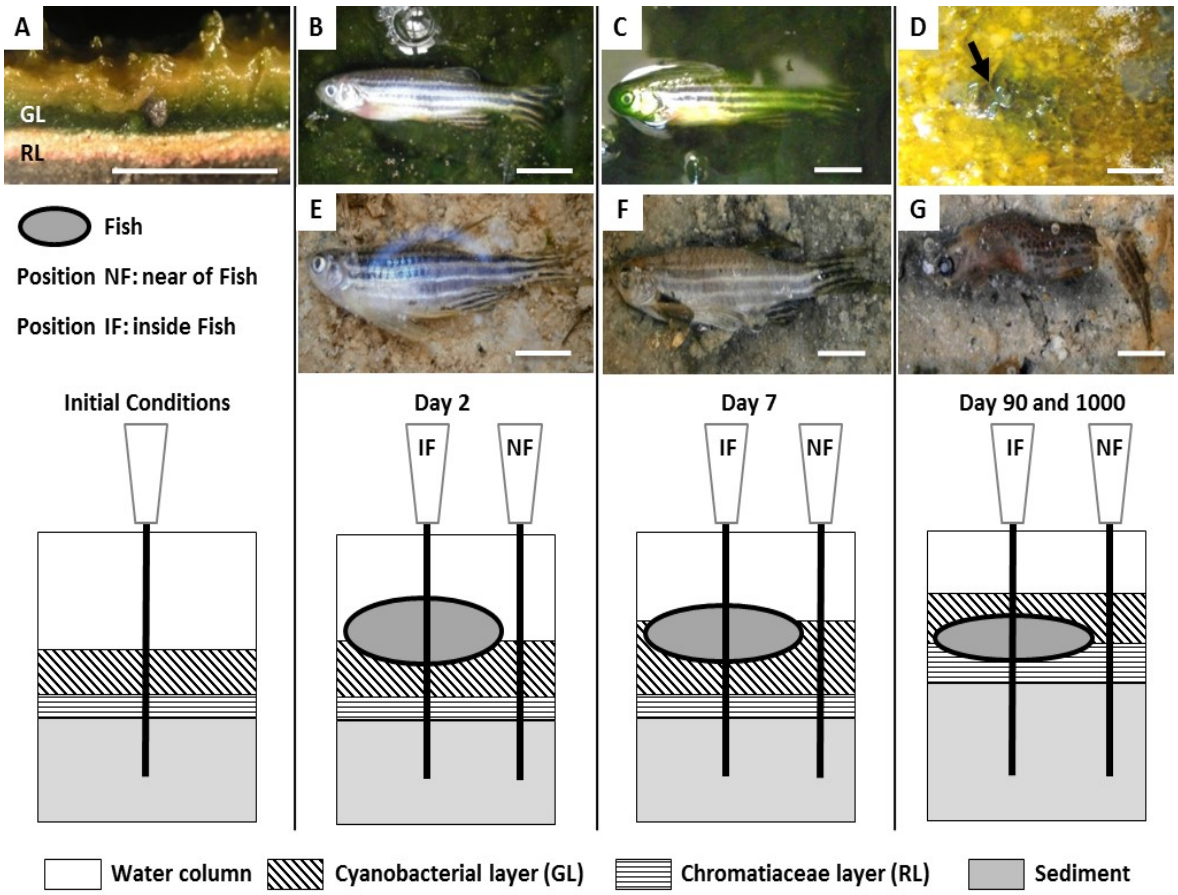

Fig. 5.1: Photographic sequence of fish at different stages of the experiment and relative positions of probes. (IF: inside fish and NF: near fish). (**A**) Transverse slice of the microbial mat at time zero before laying fish corpses. (**B-C-D**) Microbial mat coverage after 2, 7 and 90 days. Following day 7 (**C**) the fish carcasses are already being trapped by cyanobacteria filaments. (**D**) Fish completely covered by the microbial mat. The arrow points to the fish's position. (**E-F-G**) Days 2, 7 and 90 of the control sediment. Extensive decay is observed during the first week (**F**) and after 90 days animals have been reduced to disarticulated remains (**G**).

Data was analysed using the XLSTAT 2014 statistical analysis add-in tool for MS Excel. The non-parametric data was tested using the Mann-Whitney-Wilcoxon test (NF vs IF, or sediment vs microbial mat tank). The Kruskal-Wallis test was used to compare differences in variables with three or more groups (i.e., different times for each experimental point and each substrate). Tukey's range test was carried out to test the

significance of the pairwise comparisons. In all of the studied cases in which variables were not related, results were considered to be significant for p-values < 0.01.

Decay of fish carcass was checked over the course of the experiment by removing samples from within their intact microbial envelope at 90 and 1,000 days after the start of the experiment. Samples were imaged using a Bruker BMT 47/40 MRI Magnetic Resonance Imaging (MRI) scanner; this non-destructive technique provides images in which the contrast differentiates between soft tissues on the basis of the differential magnetic excitation of the protons contained in water. The relative energy gained causes the more hydrated tissues to produce lighter images compared to those tissues containing less water. In addition, the microbial layer that covered every carcass was also observed by Scanning Electronic Microscopy (SEM). Observations were carried out at the Institut de Minéralogie et de Physique des Milieux Condensés (IMPMC, Paris) using a Zeiss Ultra 55 SEM (Carl Zeiss AG, Oberkochen, Germany) equipped with a field emission gun. Images were acquired with the microscope operating at 15 kV, and a working distance of ~7.5 mm in the backscattered electron mode using the AsB detector. Samples were gold-coated prior to SEM inspection.

RESULTS

After the deposition of carcasses, an important reorganization of the structure of the mat was observed, especially within the layers made up of phototrophic bacteria, i.e., the green layer constituted by cyanobacteria and the red layer formed by anoxygenic photosynthetic bacteria. Over the course of the first two days, the mat under each fish began to turn dark green indicating an accumulation of photosynthetic pigments due to localized stimulation of cyanobacterial activity. Growth of the mat resulted in the carcass being covered completely by cyanobacteria after day 10. Subsequently, the red layer grew upwards until it reached the fish (Fig. 5.2A). The covering layer then developed until a thick coat of microbial mat rich in exopolymeric substances (EPS) (Fig. 5.2B) generated a three-dimensional sarcophagus that protected the body.

Evolution of Oxygen Profiles

Next to Fish on the Microbial Mat (Fig. 5.3). — Initially, the dissolved oxygen increased downwards from the surface of the water column and reached a maximum of 570 µM just under the mat surface (Fig. 5.3A). Below the DO maximum, the oxygen levels began to decrease: considerable reduction occurred throughout the red layer and total anoxia was reached just below of the sediment layer's top. Two days after deposition of the fish, DO showed a substantial decrease spanning from a maximum (449 µM) close to the mat surface (less than 0.5 mm from the top) to a minimum (0 µM) just above the sediment (Fig. 5.3B). After seven days the DO increased (539 µM) (Fig. 5.3C), although it was still below its original baseline value. After three months (Fig. 5.3D, day 90) the DO reached its greatest observed value (632 µM) just below the surface of the mat. After three years (Fig. 5.3E, 1000 days), the mat's oxygen level was low overall, highest in the overlying water, and no distinct peak in DO was observed.

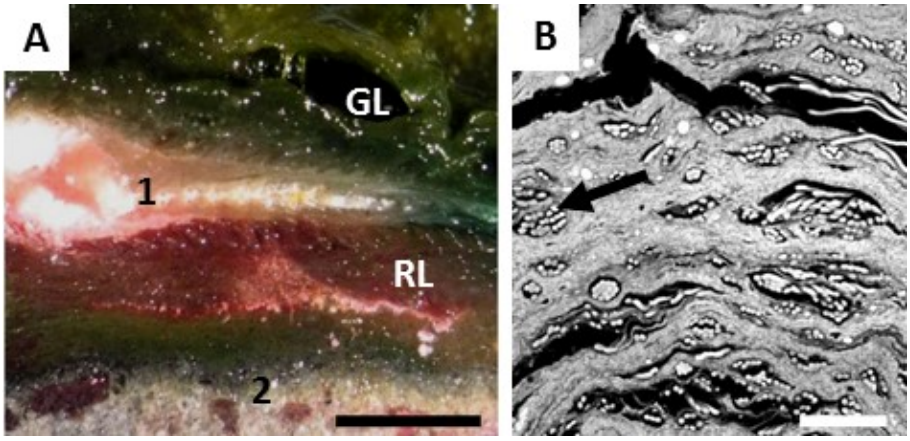

Fig. 5.2: Coronal cut of the fish completely covered by the microbial mat after 240 days. (**A**) Fish tail. Layers of the microbial mat: GL, green layer; RL, red layer below of the fish. 1, fish tail; 2, sediment. Scale bar represents 0.5 cm. (**B**) Scanning electron micrograph visualizing the coat matrix formed mainly by exopolymeric substances (EPS). The arrow indicates a group of *Coleofasciculus sp.* filaments residing in the EPS matrix. (Scale bar represents 50 µm)

Inside Fish on the Microbial Mat (Fig. 5.4). — The oxygen profiles inside the fish (point IF) also varied over time. At days two and seven (Fig. 5.4B and 5.4C) the profiles exhibited two high peaks in the levels of oxygen: one in the water column just above of the carcass (540 and 670 µM, respectively), and the other in the mat slightly below the fish (327 and 658 µM, respectively). Inside of the fish the DO was zero. After three months the mat had completely covered the fish (Fig. 5.1D) and the maximum DO (781 µM) was 0.5 mm below the mat's surface. The DO inside fish was lower but above zero (Fig. 5.4D). After 1,000 days (Fig. 5.4E), fish carcasses had lost half their thickness (Fig. 5.5C and 5.5D) compared to their initial volumes (Fig. 5.5A). Most of this reduction was achieved before day 90 and was limited after this (Fig. 5.5B). In this last stage (Fig. 5.4E) the DO profiles resembled those found at sites next to the carcasses (point NF, Fig. 5.3E) with low values overall that reached zero inside the fish bodies.

For each of the datasets inside (IF) and near (NF) the fish, the changes over time in the DO profile were remarkable and statistically significant (Table 5.1, rows 1 and 5). For the IF dataset, for days 2 and 7 there is no statistically significant difference between days

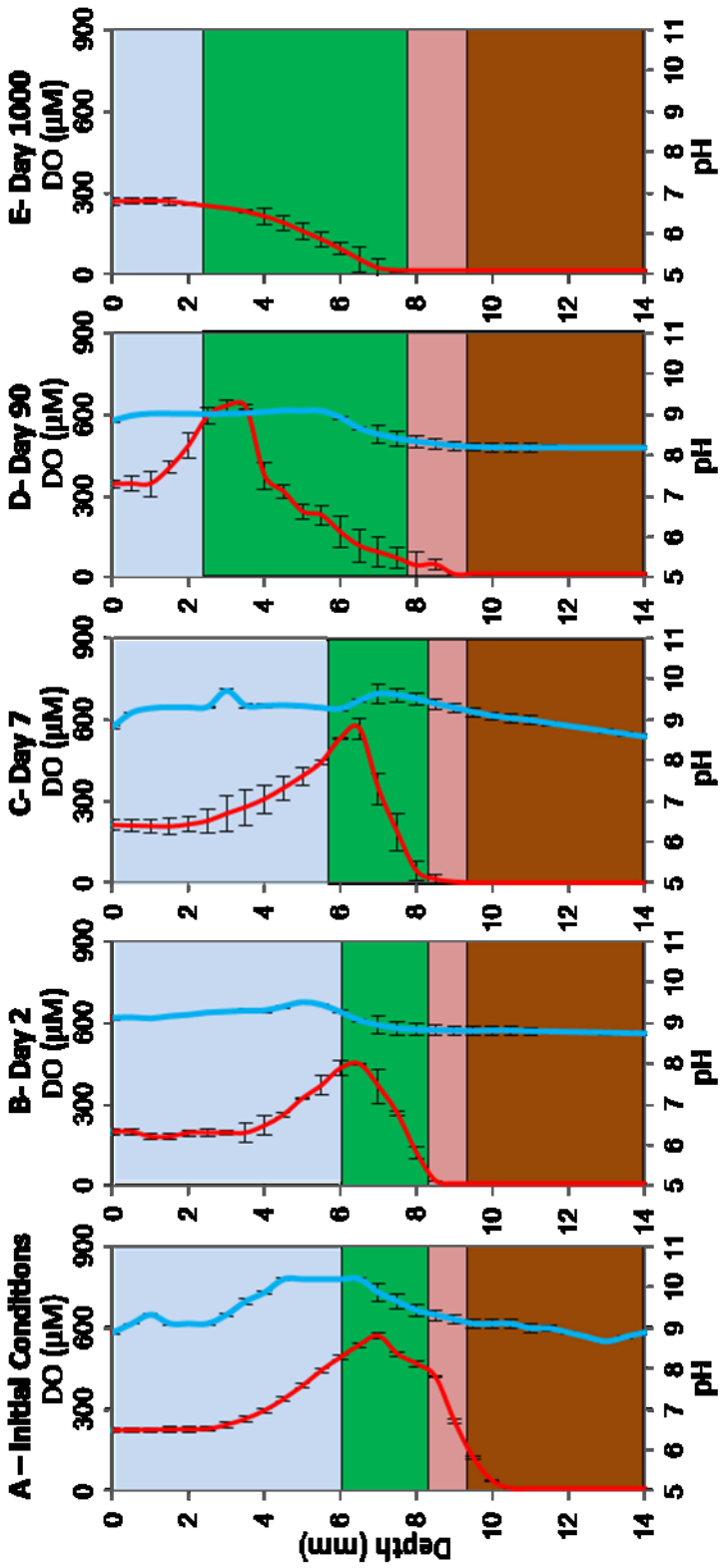

Fig. 5.3: Dissolved oxygen (DO) and pH profiles recorded at point NF (next to fish), before carcass deposition (A), and 2 days (B), 7 days (C), 90 days (D) and 1,000 days (E) after initiation of experiment. During the first week, despite reduced DO and pH compared to initial conditions, the system remained still oxygenated and basic. After 90 days, the DO and pH increased and exceeded initial values. By day 1000, the DO in the system was stable. Due to equipment malfunction the 1,000 day pH values are absent. Bars represent standard deviation.

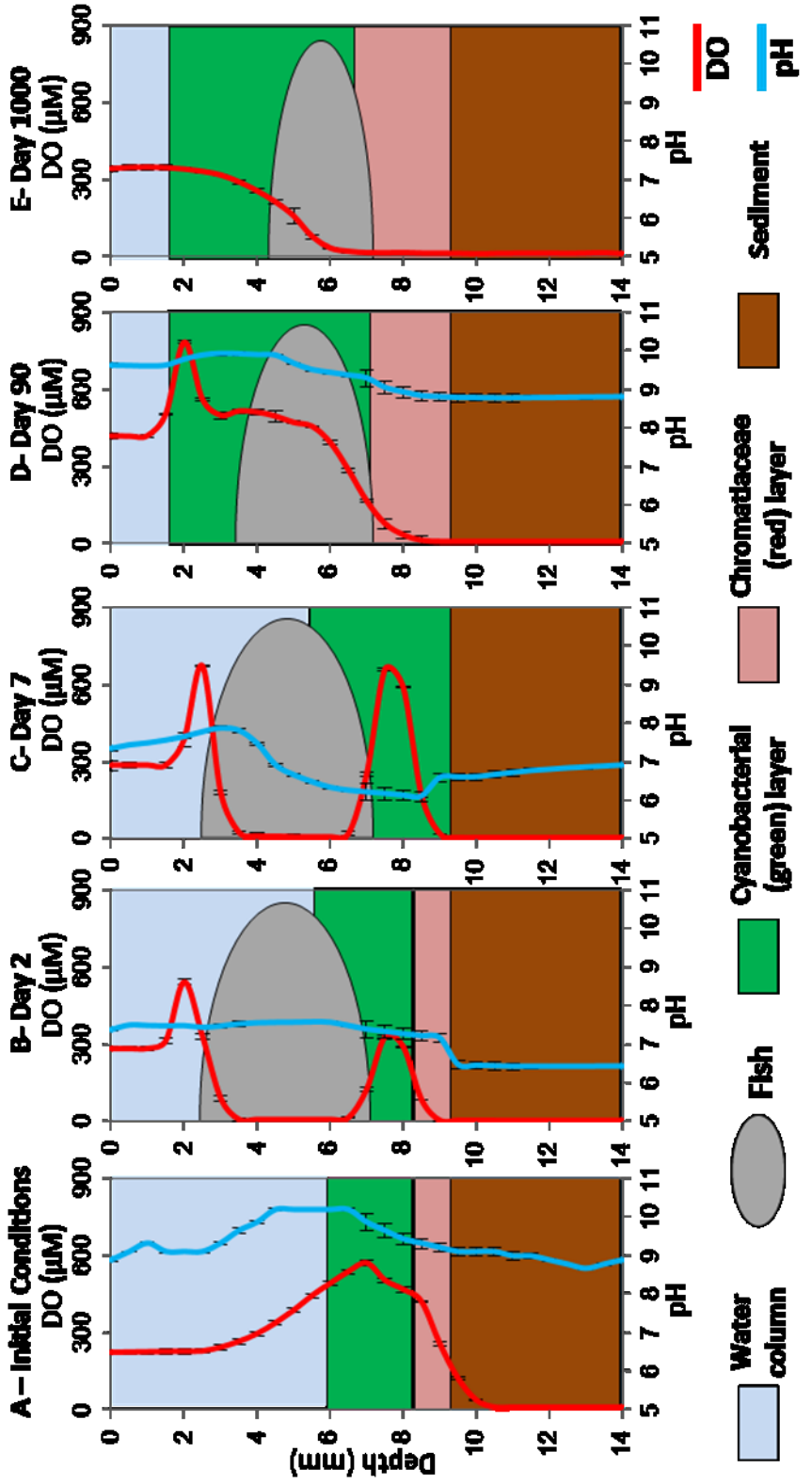

Fig. 5.4: Dissolved oxygen (DO) and pH profiles recorded at point IF (inside fish) before carcass deposition (A), and 2 days (B), 7 days (C), 90 days (D) and 1,000 days (E) after initiation of experiment. A two-stage process was observed. During the first week, the fish interiors became anoxic and reached an acidic pH caused by active decay processes. In a second phase, a slowdown in decay rate resulted in an increase in the DO concentration and pH returned to basic conditions. By day 1,000 the DO value was stable, with the fish located in the oxic zone. Due to equipment malfunction the 1,000 day pH values are absent. Bars represent standard deviation.

2 and 7: only day 90 was significantly different from all of the other days (see Supplementary Table S5.3). At point NF changes were more drastic and only the comparison between day 7 and day 90 was not significantly different (see Supplementary Table S5.1). Comparing the corresponding DO dataset for NF and IF profiles at each experimental time, only day 1,000 did not reveal significant differences (Table 5.2, rows 1 to 4).

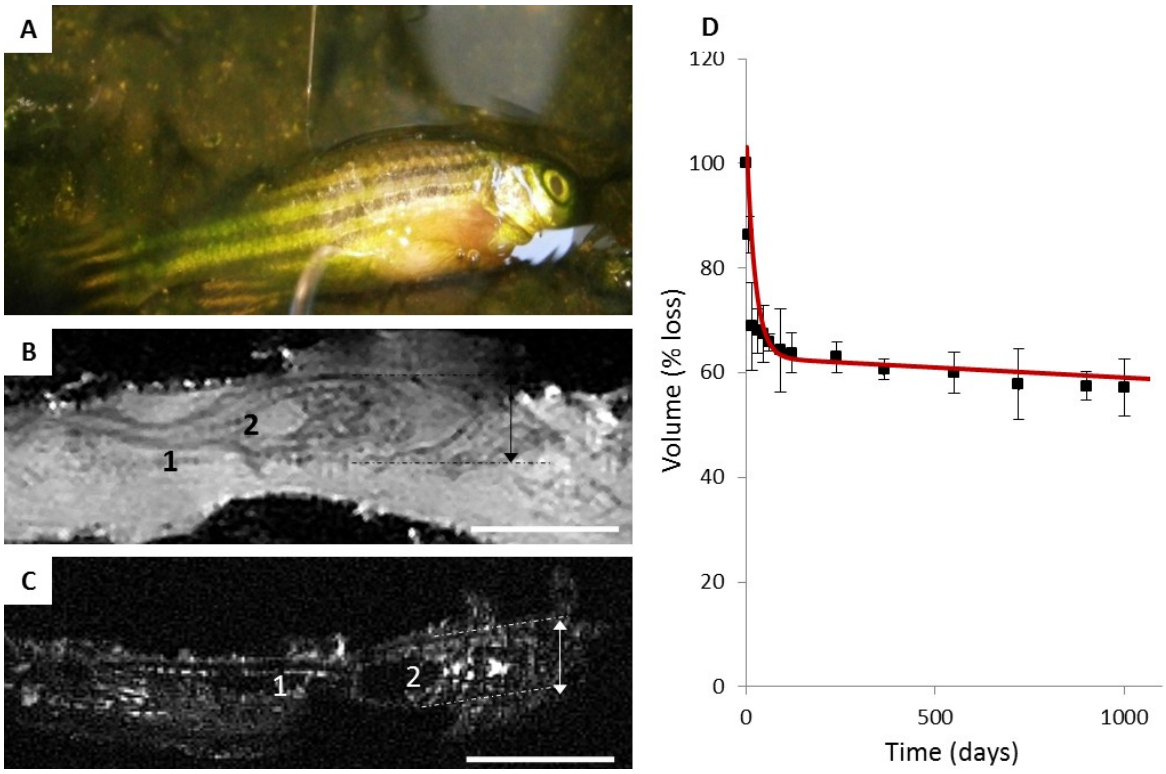

Fig. 5.5: Fish carcass thickness loss. A) Day 2 fish appearance at the moment of measurement. B and C) Volume loss analyzed by RMI (coronal view). On day 90, the fish was 4 mm thick (B), whereas on day 1,000, the fish had lost volume and its thickness had been reduced to 3 mm (C). Arrows show the measured thickness. 1: fish tail; 2: swim bladder (still preserved after 1,000 days. D) Diagram presents the progressive volume loss of fish carcasses decaying in the microbial mat. The plot also includes data derived from Iniesto et al. (2013).

Control Tank (Figs. 5.6 and 5.7). — In the control tank the DO remained close to anoxia throughout the water column except for the water's surface, where it was in contact with air. At the outset the DO decreased regularly from a maximum value (276 µM) at the water surface until reaching zero at a depth of 5.5 mm. Next to the fish bodies, DO values were even more limited, and reached zero 2 mm above the sediment surface after three months (Fig. 5.6D). Measurements taken inside the fish also revealed consistently very low values– at day two, readings ranged from the 120 µM observed for the fish surfaces to practically zero at the bottom stratum (Fig. 5.7B). On day 7, the DO was close to anoxia immediately above the bodies' surfaces (Fig. 5.7C) and by day 90 the entire bodies were anoxic (Fig. 5.7D), although the uppermost part of the water column reached a maximum of 249 µM. DO variations over the course of time proved to be significant for both the NF and IF points (Table 5.1, rows 3 and 7). Pair comparisons demonstrated that for both the NF and IF datasets, only day 0 was significantly different

from the other experimental times (Supplementary Tables S5.5 and S5.7). The DO profile comparisons inside and near to fish supported no significant differences respective to the experimental times (Table 5.2, rows 8 to 10).

Table 5.1 —Statistical analysis using the Kruskal-Wallis test to compare differences in DO and pH between the experimental times under each experimental condition (mat, control, NF or IF). Tukey's range test to check significance of pairwise comparisons furnished in the Supplementary data. Differences were considered significant (in bold) at a p-value < 0.01. *df: degrees of freedom

	Comparison between the experimental times of the differences in:		df *	F	Sig.
1	DO near the fish in microbial tank	MM_NF_DO	88	4.340	**0.000**
2	pH near the fish in microbial tank	MM_NF_pH	88	2.294	**0.000**
3	DO near the fish in control tank	SED_NF_DO	48	6.337	**0.000**
4	pH near the fish in control tank	SED_NF_pH	48	3.668	**0.000**
5	DO inside the fish in microbial tank	MM_IF_DO	118	94.895	**0.000**
6	pH inside the fish in microbial tank	MM_IF_pH	82	50.112	**0.000**
7	DO inside the fish in control tank	SED_IF_DO	64	5.939	**0.000**
8	pH inside the fish in control tank	SED_IF_pH	62	1.121	0.311

Comparison of results in experimental and control tanks. — For the corresponding experimental times, the DO profiles are statistically different between the mat and control tanks for both the IF and NF measurements (Table 5.3, rows 1-3 and 7-9). In all of the above cases the DO values were lower in the control than the microbial mat tank (Fig. 5.3, 5.4, 5.6 and 5.7).

Evolution of pH profiles

Next to Fish on the Microbial Mat (Fig. 5.3). — The microbial mat's initial profile was basic, with values from a pH of 10.2 just above the mat's photosynthetic surface to a pH of 8.7 in the sediment (Fig. 5.3A). The presence of the fish carcass resulted in a slight pH reduction which nonetheless remained basic throughout the profile (Fig. 5.3B-D). The minimum pH value observed corresponded to the sediment after 90 days (pH 8.2, Fig. 5.3D).

Inside Fish on the Microbial Mat (Fig. 5.4). — The high alkalinity observed before the fish carcasses were placed into the tank (Fig. 5.4A) contrasted with several pH readings obtained subsequently from inside the fish. The greatest fluctuations in pH occurred during the first week as the profiles became progressively more acidic (Figs. 5.4B and 5.4C). The pH values on the second day were close to neutral inside the fish carcasses (pH 7.4) and slightly acid (pH 6.4) in the sediment. On day seven the pH profile was more variable; the pH decreased further inside the fish (pH 6.9) and its minimum value (pH 6.1) was recorded in the red layer, below which the pH began to slowly rise with depth (Fig. 5.4C). After 90 days, the pH values increased, reaching their highest value in the superficial mat and then decreasing through the carcass and down into the sediment, after which the values are constant with depth (Fig. 5.4D).

Table 5.2 —Statistical analysis using the Mann-Witney Wilcox test to compare differences in DO and pH between the two points analyzed (NF and IF) at each experimental time for the microbial mat and the control sediment. Differences were considered significant (in bold) at a p-value < 0.01. *df: degrees of freedom

	Comparison between the two points analyzed (NF and IF) of the differences in:		df *	F	Sig.
1	DO in mat tanks at day 2	MM_DO_2d	22	18.104	**0.000**
2	DO in mat tanks at day 7	MM_DO_7d	22	24.200	**0.000**
3	DO in mat tanks at day 90	MM_DO_90d	22	15.263	**0.000**
4	DO in mat tanks at day 1000	MM_DO_1000d	22	2.030	0.013
5	pH in mat tanks at day 2	MM_pH_2d	22	1.437	0.140
6	pH in mat tanks at day 7	MM_pH_7d	22	7.133	**0.000**
7	pH in mat tanks at day 90	MM_pH_90d	22	33.988	**0.000**
8	DO in control tanks at day 2	SED_DO_2d	16	1.174	0.329
9	DO in control tanks at day 7	SED_DO_7d	16	1.318	0.234
10	DO in control tanks at day 90	SED_DO_90d	16	2.186	0.023
11	pH in control tanks at day 2	SED_pH_2d	16	0.360	0.984
12	pH in control tanks at day 7	SED_pH_7d	16	5.537	**0.000**
13	pH in control tanks at day 90	SED_pH_90d	16	0.879	0.596

Statistical analysis revealed the differences between the profiles at different experimental times are significant for both the NF (Table 5.1, row 6) and IF (Table 5.1, row 2) datasets (see Supplementary Tables S5.2 and S5.4 for comparison by pairs). Comparison between the NF and IF measurements (Table 5.2, row 5-7) disclosed that differences were significant except for day 2 (p-value = 0.140).

Table 5.3 —Statistical analysis using the Mann-Witney Wilcox test to compare differences in DO and pH between the microbial mat and the control sediment with respect to experimental times and fish distances (IF or NF). Differences were considered significant (in bold) at a p-value < 0.01. *df: degrees of freedom

	Comparison between the microbial mat and the sediment control of the differences in:		df *	F	Sig.
1	DO near the fish at day 2	NF_DO_2d	16	9.219	**0.000**
2	DO near the fish at day 7	NF_DO_7d	16	5.543	**0.000**
3	DO near the fish at day 90	NF_DO_90d	16	3.820	**0.001**
4	pH near the fish at day 2	NF_pH_2d	22	1.077	0.401
5	pH near the fish at day 7	NF_pH_7d	22	5.936	**0.000**
6	pH near the fish at day 90	NF_pH_90d	16	3.166	**0.002**

7	DO inside the fish at day 2	IF_DO_2d	22	17.335	**0.000**
8	DO inside the fish at day 7	IF_DO_7d	22	75.298	**0.000**
9	DO inside the fish at day 90	IF_DO_90d	22	344.324	**0.000**
10	pH inside the fish at day 2	IF_pH_2d	16	0.619	0.849
11	pH inside the fish at day 7	IF_pH_7d	16	0.284	0.996
12	pH inside the fish at day 90	IF_pH_90d	20	119.970	**0.000**

Control Tank (Figs. 5.6 and 5.7). — The variation in the pH profiles of the IF measurements over time were not statistically significant; those for the NF data were (Table 5.1, rows 8 and 4 respectively). Pairwise comparisons indicated that the differences between experimental times were significant with in the case of both the IF and NF (except for days 2 and 7, Supplementary Tables S5.6 and S5.8). Profile variations of points NF and IF at different times were significant except for day 7 (Table 5.2, row 12), which was slightly more acidic near the fish.

Comparison of corresponding results in experimental and control tanks. — The differences between the control and mat tanks near to the fish were consistently significant (except for day 2; Table 5.3, row 4); when significantly different, the values recorded in the case of the microbial mat were more basic. Inside of the fish, the profiles were not significantly different at either days 2 and 7 (Table 5.3, rows 10 or 11). On day 90, the pH of the mat returned to a basic condition and was significantly different from the control (Table 5.3, row 6 and 12).

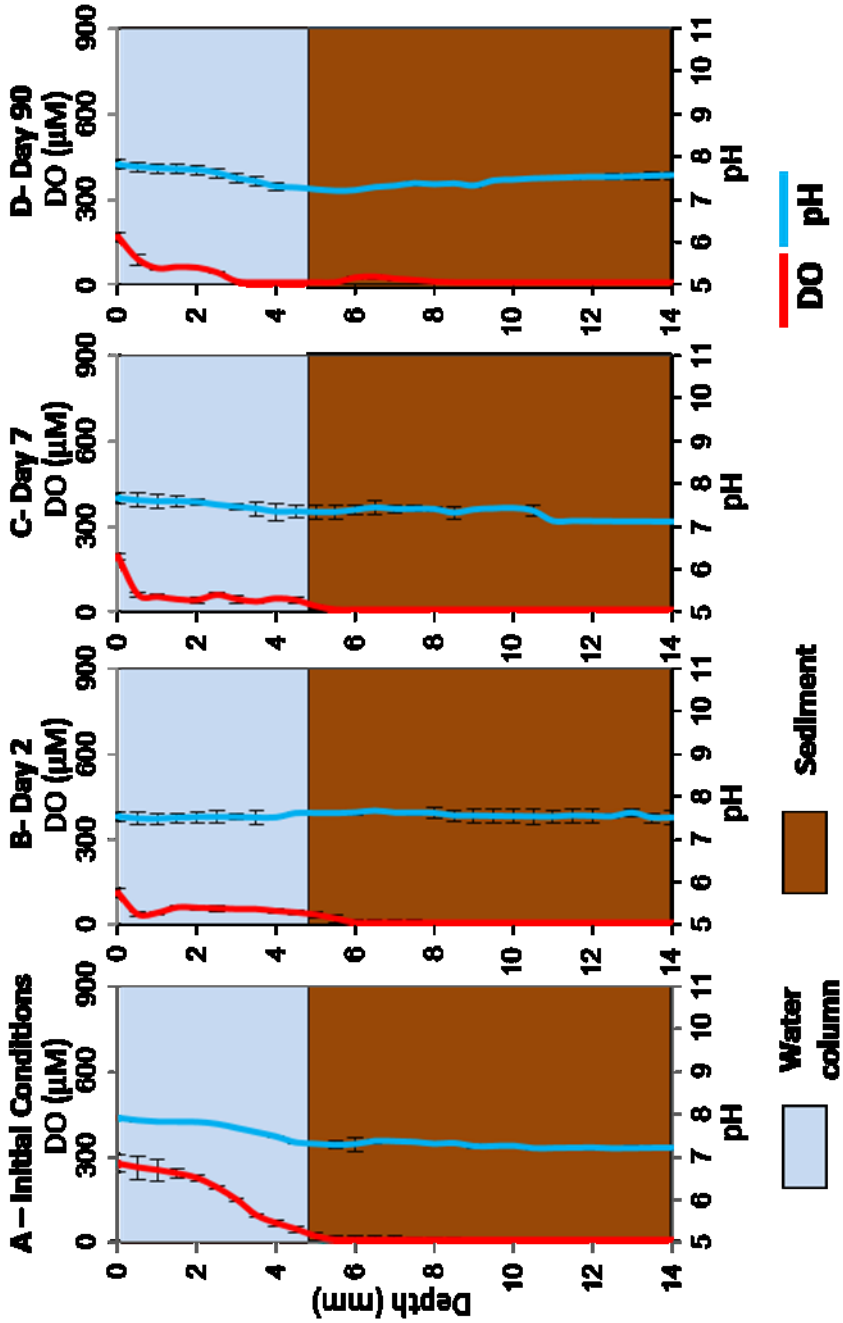

Fig. 5.6: Dissolved oxygen (DO) and pH profiles recorded at point NF (next to fish) in the control sediment before carcass deposition (A), and 2 days (B), 7 days (C) and 90 days (D) after initiation of experiment. Initially DO level decrease regularly from the water column surface until attaining anoxia at the sediment surface. The DO concentration remained close to anoxia except for the water surface in contact with air all the way through the experiment. Notice that on day 90 the DO level further decreased becoming zero at 2 mm above of the sediment's surface. Bars represent standard deviation.

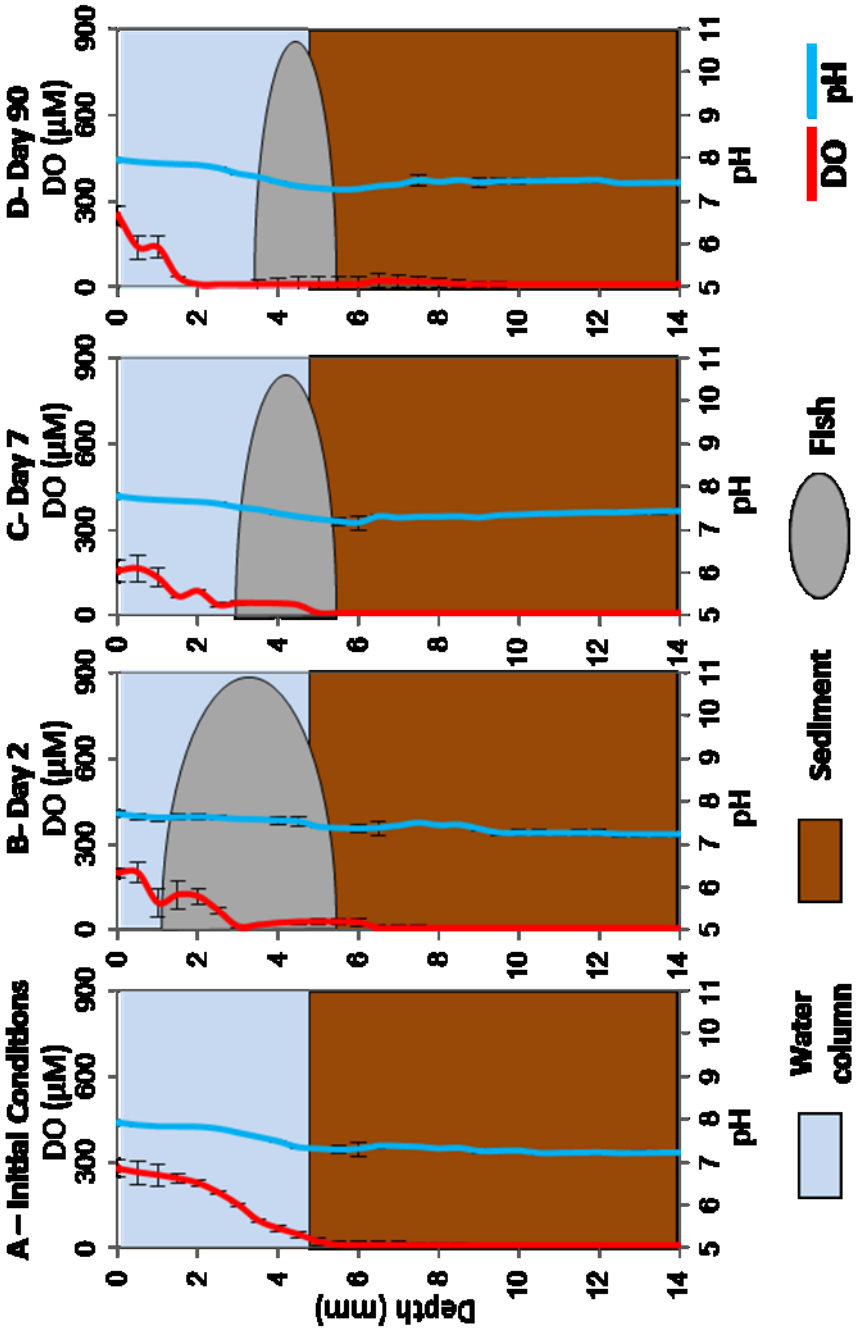

Fig. 5.7: Dissolved oxygen (DO) and pH profiles recorded at point IF (inside fish) in the control sediment before carcass deposition (A), and 2 days (B), 7 days (C) and 90 days (D) after initiation of the experiment. After 1,000 days the control fish decay was complete and profile determinations were not performed. Measurements made inside fish disclosed a very low DO concentration and a slightly acidic pH. Despite of these conditions extensive decay was observed. Bars represent standard deviation.

DISCUSSION

It has long been recognised that taphonomy experiments are decisive in order to reproduce the original physicochemical conditions that are likely to have developed of the early stages of fossilization. We describe here the chemical conditions generated on a millimetric scale by the microbial mats after the deposition of fish carcasses.

Decomposition of the fish was influenced by the microbial mat in two key steps, which were absent in the control tank. Stage one occurred during the first week. Drops in DO next to the fish could either have been the result of the inhibition of the photosynthetic activity or an increase in the respiration rate of the microorganisms involved in the aerobic decomposition of the carcasses sufficient to mask the on-going production of oxygen. Inhibition of photosynthesis seems the less likely as the mat became activated around of the carcasses and the mat's growth rate increased quickly, trapping fish bodies entirely. In addition, the pH next to the carcasses remained basic, which is a direct consequence of the increase of the photosynthetic activity. Furthermore, the mat's activity would have been stimulated by the release of organic and inorganic nutrients into the environment from decomposition of the body. Inside the fish, this first phase was characterized by anoxia, and despite the extensive DO variation, the pH did not display any relevant acidification trend until after day 7. The DO underwent a rapid and significant reduction caused by depletion of oxygen by heterotrophic aerobic respiration and the pH profile changed from basic to slightly acidic. Under these conditions, the microorganisms responsible for decomposition could either ferment or use anaerobic respiration pathways, leading to a pH decrease in response to the release of acid compounds. Throughout these first days, decomposition processes were active as demonstrated by the noticeable loss of thickness of the body (see Fig. 5.5D and Iniesto et al. 2013).

Most importantly, the variations in pH and DO are decoupled during the first stage both temporally and spatially. The inside of the fish is anoxic while DO is high above and below the body, whereas pH remained essentially stable through the vertical profile. While DO maintained the same pattern, the pH progressively decreased inside the carcass, especially at the possibility of lower surface (see Fig. 5.3B and 5.3C). Such decoupled chemical conditions hint towards a differential preservation between the upper and lower carcass surfaces.

The second phase corresponds to day 90, when substantial changes were observed in the surroundings and inside the fish: a DO recovery exceeding its initial value (maximum reached above the fish) in conjunction with a pH return to the original basic condition. After the initial period of decay, what remains of the carcass is more recalcitrant and therefore decomposition proceeds slower, as implied by the decrease in the rate at which the thickness of the specimens decreased (Figure 5.5D). The decrease in the rate of microbial consumption of oxygen was balanced by the rate of oxygen production by photosynthesis and resulted in the oxygenation inside of the fish. During this second phase, the oxic environment would drastically limit the anaerobic decomposers'

activities in the upper layers of the mat. As a result of the slower local release of acids and the increase in photosynthesis, the pH would rise. At this point in the presence of the microbial mat, preservation mechanisms would mainly depend on high pH values and oxic conditions, driven mainly by the activity of cyanobacteria. In the course of the experiment, the system loses activity and dynamism as nutrients are depleted, resulting in lower oxygen production and consequent mat stabilization.

The results presented here investigates a more complex system compared to previous decay experiments (e.g. inoculation with a heterotrophic bacteria veil, such as Sagemann et al. 1999) by incorporating the effect of an actual microbial mat (i.e., with photosynthetic autotrophic aerobic and anaerobic microorganism communities). Furthermore, the experiment's long duration (3 years) has revealed a novel phase of the process, which follows the anaerobic conditions previously described during decay (Briggs and Kear 1993; Raff et al. 2008). It has been long assumed that low pH and anoxia are conditions that favour authigenesis (Sagemann et al. 1999) but they seem not to be the only ones for preservation in mats according to our results. In particular, the interior of the fish placed in the control tank remained anoxic throughout of the experiment and the pH was significantly lower than in the mat tank. In spite of this, controls decomposed substantially. The decay of fish covered by mats was slowed down to such an extent that even some soft structures (skin, muscles and tendons) were retained over a longer period of time (see Fig. 5.2A and 5.5, and Iniesto et al., 2013). Therefore, major differences in preservation occurred during the second phase, when DO and pH conditions are driven by the oxygenic phototrophic microorganisms, and the system as a whole is oxic with a basic pH value.

The role played by microbial communities in inducing mineralization in microbial mats (bioprecipitation) is complex. Some microbial activities of the mats, such as aerobic (Visscher et al. 1998) or anaerobic (Vasconcelos et al. 2006) photosynthesis, sulphate reduction or even aerobic carboxylic acid degradation can lead to a pH increase and carbonate precipitation (Dupraz and Visscher 2005). The organic EPS matrix entails a two-fold role being equally involved in carbonate formation and degradation, and its role varies depending on environment-specific physicochemical and biological features (Dupraz et al., 2009). This matrix can work as a cation chelator, removing in consequence Ca from the environment (Dupraz and Visscher 2005). However, when the EPS matrix undergoes biological degradation, the Ca^{2+} is released, favouring carbonate precipitation (Dupraz and Visscher 2005), with the matrix acting as a template that drives crystal nucleation (Costerton et al. 1995). As a result of the localized $CaCO_3$ precipitation within mats, lithification of layers can occur (Decho et al. 2005). Our results elucidate some of the relationship between the microenvironmental changes and the bioprecipitation process. The inside of the fish remains anoxic from the outset; two days after the fish was deposited on the mat, anoxia is accompanied by a pH decrease throughout the entire profile. By contrast, outside the fish DO decreases (most probably as a result of heterotrophic aerobic activity) but the pH remains basic (pH ~9). The growth of heterotrophic microorganisms may be limited by nutrient availability as fish scales are difficult to degrade and contain little N. When this happens, heterotrophic

bacteria can degrade the EPS matrix made up mainly of lipids and carbohydrates (Sand and Gehrke 2006) in order to obtain needed nutrients. Oxygenic photosynthesis is on-going but masked by oxygen consumption due to aerobic respiration and it explains the high pH outside of the fish. The addition of a source of Ca^{2+} (e.g. via the degradation of the EPS matrix) would create the necessary conditions for carbonate precipitation as suggested by Iniesto et al. (2013), who observed a Ca-enriched film on a fish decayed for 15 days.

In the longer term, after being covered by microbial mats, the fish interior become oxic with high pH values, especially three months after deposition. These conditions during the second phase are different from the role of heterotrophic biofilms in fossilization described by Wilby et al. (1996). Indeed, the taphonomic experiments conducted by Briggs et al. (1993), suggested that the shift between carbonate and phosphate is linked to pH conditions, allowing and favouring the preservation of soft-tissues. However, although Briggs and Wilby (1996) linked the occurrence of calcium phosphate to a decrease in pH, the precipitation of this mineral phase in basic environments is well known (Benzerara et al. 2004; and Recillas et al. 2012). Indeed, Song et al. (2002) found that the optimal pH for calcium phosphate precipitation in experiments (at lower-moderate concentrations of phosphate, consistent with our system) ranged from 8.5 to 9 depending on the Ca:P ratio (see in particular Fig. 2 and Fig. 3 in Song et al. 2002). Under these high-pH conditions, the switch between phosphate and carbonate would depend on the concentration of P and the Ca:P ratio in the system, rather than on pH (Bachra et al. 1963). In addition, other minerals such as magnesium silicates (Souza-Egipsy et al. 2005) are also favoured by this oxic-basic phase. Therefore, further studies are still necessary to contrast whether mineral replication of soft tissues should only take place in the course of early decay (anoxic and acid phase), or if mineralization (e.g., calcium phosphate, magnesium silicates) might occur in the oxic and basic phase of the microbial mat in the long-term, as plausible evidence suggests.

The experiment has confirmed that in the process of covering carcasses on their surface, microbial mats are responsible of generating complex chemical microenvironments that inhibit decomposition and increase preservation of tissues. In contrast, when the microbial mat is absent, fish exhibit active, persistent and continual decay. The mat's role was twofold. During the first stage, DO underwent a significant reduction and the pH turned slightly acidic inside the fish. On the outside of the fish carcasses, the pH remained basic and would allow the precipitation of several mineral phases such as silicates, carbonates and phosphates, depending on the elements released by the degradation of EPS. This short-lived phase has been detected previously in experimental studies (e.g. Sagemann et al. 1999). During the second phase, the inside of the fish became oxic and the pH was basic and the remains' soft inner tissues were preserved. The microbial coverage and the subsequent protective microenvironment would potentially be able to promote pseudomorphism and biomineralization processes, explaining the exceptional preservation of carcasses within microbial mats.

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SUPPLEMENTARY TABLES S5.1 AND S5.2

Supplementary Tables S5.1 and S5.2: Comparison by pairs checking differences between experimental times in microbial mat tank in DO (Table S5.1) and pH (Table S5.2) (Tukey Test) near carcasses (point NF)

Table S5.1: DO

T1	Vs T2	Sig.
0	2	**0,000**
	7	**0,001**
	90	**0,008**
	1.000	**0,005**
2	0	**0,000**
	7	**0,010**
	90	**0,007**
	1.000	**0,000**
7	0	**0,001**
	2	**0,010**
	90	0,999
	1.000	**0,000**
90	0	**0,008**
	2	**0,007**
	7	0,999
	1.000	**0,000**
1000	0	**0,005**
	2	**0,000**
	7	**0,000**
	90	**0,000**

Table S5.2: pH

T1	Vs T2	Sig.
0	2	**0,002**
	7	**0,002**
	90	**0,007**
2	0	**0,002**
	7	0,000
	90	**0,000**
7	0	**0,002**
	2	0,000
	90	**0,000**
90	0	**0,007**
	2	**0,000**
	7	**0,000**

SUPPLEMENTARY TABLES S5.3 AND S5.4

Supplementary Tables S5.3 and S5.4: Comparison by pairs checking differences between experimental times in microbial mat tank in DO (Table S5.3) and pH (Table S5.4) (Tukey Test) inside carcasses (point IF)

Table S5.3: DO

T1	Vs T2	Sig.
0	2	**0,000**
	7	**0,000**
	90	0,805
	1.000	0,111
2	0	**0,000**
	7	0,015
	90	**0,000**
	1.000	0,805
7	0	**0,000**
	2	0,015
	90	**0,000**
	1.000	0,111
90	0	0,805
	2	**0,000**
	7	**0,000**
	1.000	**0,000**
1000	0	0,111
	2	0,805
	7	0,111
	90	**0,000**

Table S5.4: pH

T1	Vs T2	Sig.
0	2	**0,001**
	7	**0,003**
	90	**0,006**
2	0	**0,001**
	7	0,983
	90	**0,000**
7	0	**0,003**
	2	0,983
	90	**0,000**
90	0	**0,006**
	2	**0,000**
	7	**0,000**

SUPPLEMENTARY TABLES S5.5 AND S5.6

Supplementary Tables S5.5 and S5.6: Comparison by pairs checking differences between experimental times in control tank (Test Tukey) (point NF).

Table S5.5: DO		
T1	Vs T2	Sig.
0	2	**0,000**
	7	**0,001**
	90	**0,005**
2	0	**0,000**
	7	0,61
	90	0,036
7	0	**0,001**
	2	0,61
	90	0,252
90	0	**0,005**
	2	0,036
	7	0,252

Table S5.6: pH		
T1	Vs T2	Sig.
0	2	**0,002**
	7	**0,002**
	90	**0,007**
2	0	**0,002**
	7	0,599
	90	**0,000**
7	0	**0,002**
	2	0,599
	90	**0,000**
90	0	**0,007**
	2	**0,000**
	7	**0,000**

SUPPLEMENTARY TABLES S5.7 AND S5.8

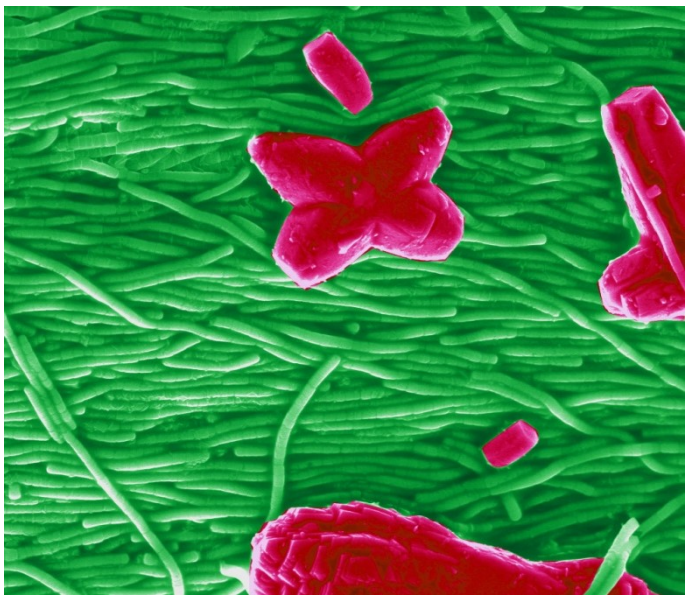
Supplementary Tables S5.7 and S5.8: Comparison by pairs checking differences between experimental times in control tank (Test Tukey) (point IF).

Table S5.7: DO		
T1	Vs T2	Sig.
0	2	**0,009**
	7	**0,006**
	90	**0,004**
2	0	**0,009**
	7	0,957
	90	0,368
7	0	**0,006**
	2	0,957
	90	0,236
90	0	**0,004**
	2	0,368
	7	0,236

Table S5.8: pH		
T1	Vs T2	Sig.
0	2	**0,001**
	7	**0,003**
	90	**0,006**
2	0	**0,001**
	7	0,955
	90	**0,001**
7	0	**0,003**
	2	0,955
	90	**0,003**
90	0	**0,006**
	2	**0,001**
	7	**0,003**

CAPÍTULO 6

PRESERVATION IN MICROBIAL MATS: MINERALIZATION BY A TALC-LIKE PHASE OF A FISH EMBEDDED IN A MICROBIAL SARCOPHAGUS



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ABSTRACT

Microbial mats have been repeatedly suggested to promote early fossilization of macroorganisms. Yet, experimental simulations of this process remain scarce. Here, we report results of 5 year-long experiments performed on fish carcasses to document the influence of microbial mats on mineral precipitation during early fossilization. Carcasses were initially placed on top of microbial mats. After two weeks, fish became coated by the mats forming a compact sarcophagus, which modified the microenvironment close to the corpses. Our results showed that these conditions favoured the precipitation of a poorly crystalline silicate phase rich in magnesium. This talc-like mineral phase has been detected in three different locations within the carcasses placed in microbial mats for more than 4 years: 1) within inner tissues, colonized by several bacillary cells; 2) at the surface of bones of the upper face of the corpse buried in the mat; and 3) at the surface of several bones such as the dorsal fin which appeared to be gradually replaced by the Mg-silicate phase. This mineral phase has been previously shown to promote bacteria fossilization. Here we provide first experimental evidence that such Mg-rich phase can also be involved in exceptional preservation of animals.

Keywords: Mg-silicate biomineralization, fish preservation, fossilization, microbial mat, experimental taphonomy

RESUMEN

La presencia de tapetes microbianos es considerada de forma generalizada como un factor que favorece la fosilización temprana de macroorganismos. Sin embargo, los experimentos que tratan de reproducir este fenómeno son escasos. En el presente estudio se muestran los resultados de experimentos llevados a cabo con cadáveres de peces durante 5.5 años, con el objetivo de documentar la influencia de estas comunidades microbianas en la mineralización durante las etapas tempranas de fosilización. Los cuerpos fueron depositados en la superficie de tapetes microbianos, que los recubrieron en 2 semanas, formando a su alrededor un compacto sarcófago que modificó las condiciones ambientales en torno al cadáver. Nuestros resultados muestran que las nuevas condiciones generadas por el tapete favorecen la precipitación de una fase de silicato rica en magnesio poco cristalizada. Este mineral, de composición similar a la del talco, fue detectado después de 4 años en tres localizaciones diferentes del sarcófago: 1) en los tejidos internos del cuerpo, que han sido colonizados parcialmente por bacterias; 2) sobre la superficie de los huesos de la cara superior de los peces y; 3) en estructuras óseas como los hemitricos de la aleta dorsal del pez, los cuales parecen estar siendo reemplazados gradualmente por esta fase de Mg-silicato. Aunque previamente había sido mostrado que dicha fase mineral podía promover la fosilización de bacterias, aquí se muestra la primera evidencia experimental de que también pueden estar involucrados en la preservación excepcional de animales.

Palabras clave: biomineralización de silicato rico en Mg, preservación de pez, fosilización, tapetes microbianos, tafonomía experimental

INTRODUCTION

The role of microbial mats in fossilization has been inferred based on several lines of evidence. First, microbial communities can remodel sediments, generating rare but clearly distinguishable features that have been named microbially-induced sedimentary structures (MISS) (Gerdes et al. 1993; Hagadorn and Bottjer 1997; Noffke et al. 2001; Noffke 2009). MISS have favoured preservation of body impressions or the formation of death masks, such as those found in the Ediacaran period (Gehling 1999; Narbonne 2005; Laflamme et al. 2011), or footprints of Late Jurassic sauropods (Marty et al. 2010). In addition, the frequent association of bacterial cells with exceptionally preserved fossils supports the link between fossilization and microbial mats. For example, the coverage of fossils of macroorganisms by fossil microbial cells has been detected in sites of different ages, belonging to diverse Konservat-Lagerstätten such as, for instance, Las Hoyas (Cretaceous, Spain) (Briggs et al., 1997; Gupta et al. 2008) or Nuspligen (Jurassic, Germany) (Briggs et al. 2005). This preserved association of microbial cells with fossils has also been observed in some pseudomorphs, replicating original tissues: for example, this is the case of the eye of a teleostean fish from Las Hoyas (Gupta et al. 2008) or several dinosaurian fossil bones of different ages (Kaye et al. 2008).

The precise mechanisms explaining how microbial mats favour the preservation of fossilized macroorganisms are yet to be clarified. One reason might be related to their capability to induce mineral precipitation, leading to the formation of lithified layers (Visscher et al. 1998; Dupraz and Visscher 2005; Vasconcelos et al. 2006; Dupraz et al. 2009), which may protect dead bodies from advanced degradation. Li et al. (2013) stressed on the pivotal role of microbially-induced authigenic mineralization in fossilization and it has been reported in many instances that bacterial cells are themselves preserved when mineral formation occurs within and/or at cell surfaces (Couradeau et al. 2013; Gérard et al. 2013).

Despite previous geobiological observations, there are still few experimental studies simulating fossilization of macroorganisms by microbial mats. Recently, taphonomic experiences have successfully shown the possibility of increased fossil preservation using microbial mats (Iniesto et al. 2013b; Guerrero et al. in press). For example, the role played by microbial mats in the cementation and the consequent preservation of footprints has been tested by Marty et al. (2009). The ability of mats to stabilize sediments by their cementation around carcasses and induction of authigenic mineralization has been achieved experimentally by Darroch et al. (2012). The preservation of an accurate impression and the formation of a mould have also been tested (Iniesto et al. 2013a) as well as the generation and maintenance of a microenvironment close to preserved corpses (Iniesto et al. 2015). In addition, Iniesto et al. (2013b) described a Ca-rich veil covering the upper surface of carcasses after 15 days. However, the extent of mineralization of carcasses, the identity of the involved minerals as well as their spatial distribution within the microbial sarcophagus remain poorly assessed. Since chemical conditions change significantly at the microenvironment-scale

during the early fossilization (Iniesto et al. 2015), the precipitation of diverse mineral phases may occur. However, the importance of this process in the preservation of soft tissue of carcasses inside the microbial sarcophagus requires further evidence.

Here, we report and discuss the results of a 5-year long experiment consisting in the coverage of fish carcasses by microbial mats in order to explain and better understand the mineralization process into the sarcophagus generated by microbial mats. To identify and describe the expected precipitates, we used a combination of bulk scale and micro- and nano-scale analyses, including x-ray diffraction (XRD), Raman microspectroscopy, scanning electron microscopy (SEM) (see Beyssac et al. 2003 or Bernard et al. 2008 for more frequent RAMAN applications). Focused ion beam (FIB) milling and transmission electron microscopy (TEM) allow the accurate characterization of mineral phases in these biological systems and the observation of the relations between tissues and microbial cells as shown by previous studies (e. g. Benzerara et al. 2005a, 2005b).

MATERIAL AND METHODS

Microbial mats and fish carcasses

Microbial mat samples used for the taphonomy experiment were collected from the shallow lake Salada de Chiprana (Zaragoza, Spain). The lake is fed by groundwater discharge, which provides a source of abundant magnesium sulphate (up to 700 meq.L^{-1} of SO_4^{2-} and Mg^{2+}) and sodium chloride (approximately 300 meq.L^{-1}). A more detailed water composition is provided by Guerrero et al. (1991). The salinity of the lake water is high, varying between 30 to 70% due to the changes in the volume of surface run-off which supplies the lake with abundant nutrients (Berga et al. 1994). Together with high pH (values above 9), this results in an extensive coverage by microbial mats of the shore down to a depth of 1.5 m (Guerrero et al., 1991). The "Salada de Chiprana" mats are dominated by the filamentous cyanobacterium *Coleofasciculus chthonoplastes* (Thuret ex Gomont) (Siegesmund et al. 2008) (formerly known as *Microcoleus chthonoplastes* Thuret ex Gomont), determining the coherence of the community. The taxa of the upper mat layer are mainly Chloroflexus, Coleofasciculus and Pseudoanabaena-like and the underlying darker layer is made up of dead cyanobacteria, frustules of diatom and anoxic microbial populations (Jonker et al. 2003). The "Salada de Chiprana" mats lie on detritic material, deposited by an ancient network of Tertiary paleo-channels composed of fossilized sands which now remain as ridges due to the differential erosion of the less resistant silt deposits between channels (Friend et al. 1986).

Microbial mats were ground until homogenization and inoculated in glass tanks (50x20x18 cm) over a layer of sediments collected from the same lake. The resulting community was similar to the natural one, with millimetric multi-layered structure, due to differential growth along a vertical gradient of physicochemical characteristics (Cohen 1989; Wierzchos et al. 1996). Layers were active during the course of the experiment, with oxygenic photosynthetic (mainly filamentous cyanobacteria and several diatoms) and aerobic heterotrophic microorganisms in the oxic upper layer, laid over several anoxygenic photosynthetic bacteria (Chromatiaceae and Chlorobiaceae groups), and anaerobic microorganisms (e.g. fermenters or sulphate-reducing bacteria) in the anoxic deep layer (Jonkers et al. 2003). One similar glass tank without microbial mat was used as a control. To prevent the formation of a microbial mat, we maintained this tank in the dark. In this way, cyanobacteria could not multiply and therefore they could not form the framework necessary for the build-up of mats. A large diversity of non-phototrophic bacteria likely colonized this tank but we could clearly observed that no mat was formed. The tanks were filled with water from Chiprana to obtain a one centimetre deep water column. The water losses resulting from evaporation were periodically compensated by adding sterilized distilled water. The system was illuminated (OSRAM DECOSTAR 51 TITAN) with a photoperiod of 10 h of daylight.

The small fish *Paracheirodon innessi* (neon tetra) was used as a model for macroorganisms. Six fish were laid at the surface of the microbial mat with a gap between the fish (Fig. 6.1A). Three fish were analysed after four years of experiments.

Three others were analysed after 5 years. Six additional fish were placed on control sediments in analogous tanks and kept in the dark to prevent the growth of an actual microbial mat as explained above (Fig. 6.1B). Fish were killed immediately before the experiment following the standard animal care protocol used at the Universidad Autónoma de Madrid. Fish used as controls decayed so much that they were barely recognizable after four years and could not be collected (Iniesto et al. 2013b). Structures such as bones and fins, initially hard, broke up during the handling of the sample, showing their high decay. In contrast, fish deposited at the surface of the mats were quickly covered by the microbial communities. The sarcophagus formed by the mats protected carcasses and made their decomposition much slower compared to controls (Guerrero et al. in press).

Scanning electron microscopy (SEM) and energy dispersive x-ray spectrometry (EDXS)

For electron microscopy analyses, samples of fish embedded within microbial sarcophagi and the underlying sediments were fixed in glutaraldehyde (2%) overnight and dehydrated in a gradual series of ethanol baths (50%, 70% and 100%). Once dried, samples were embedded in epoxy resin, sectioned with a diamond saw blade along a coronal plane (Fig. 6.1C) and polished with diamonds down to ¾ µm in size. Samples were then coated with carbon. Images and analyses were collected in backscattered and secondary electron modes using a Zeiss Ultra 55 FEG-SEM operating at 15 kV, with a 60 µm aperture at a working distance of ~7.5 mm. Elemental compositions were determined using energy dispersive x-ray spectrometry (EDXS) with a Bruker QUANTAX system. EDXS maps were processed using the software Esprit 1.9 (BRUKER).

Focused ion beam (FIB) milling and scanning transmission electron microscopy (STEM)

Several areas observed by SEM were selected to excavate electron-transparent foils by focused ion beam (FIB) milling. These foils were then be analysed down to the nm-scale by scanning transmission electron microscopy (STEM) similarly to numerous previous studies on mineral-living organism assemblages (e.g., Benzerara et al. 2005; Bernard et al. 2010). FIB milling was performed using a FEI strata DualBeam 235.

STEM observations of the ultrathin foils were carried out using a JEOL2100F (JEOL Ltd, Japan) microscope operating at 200 kV, equipped with a field emission gun and a high resolution UHR pole piece. STEM imaging allows Z-contrast imaging in the high angle annular dark field (HAADF) mode. Elemental mapping was acquired by EDXS in the STEM mode with a 1 nm probe. Selected area electron diffraction (SAED) was performed using the smallest aperture allowing retrieval of diffraction patterns from a 100 nm x 100 nm area.

X-ray diffraction (XRD) and Raman microspectroscopy

The sediment was kept overnight at 37ºC and dry-crushed until obtaining a homogeneous powder. XRD measurements were performed using the Co Kº radiation (1.7889 Å) on a Panalytical X'Pert Pro MPD diffractometer mounted in Bragg-Brentano configuration. Data were recorded over a 4-70° 2θ range with a step of 0.017º and a counting time of 3.5 h per sample. Raman microspectroscopy was performed using a Renishaw InVIA Reflex microspectrometer. Rationale for Raman analyses can be found in Bernard et al. (2008) and Beyssac and Lazzeri (2012). We used a 514 nm Laser Physics argon laser in circular polarization. The spectrometer was first calibrated with a silicon standard. The laser was focused on the sample through a DMLM Leica microscope with a 100 × objective (NA = 0.85), and the laser power at the sample surface was set around 1 mW. The Rayleigh diffusion was eliminated by edge filters, and the entrance slit was closed down to 15 µm to achieve nearly confocal configuration. The signal was finally dispersed using a 1800 lines/mm grating and analyzed by a Peltier-cooled RENCAM CCD detector.

Water Analysis

Conductivity, temperature and pH of the water column were measured once a week with the corresponding electrodes (WTW-LF 330 conductivity meter and a 323 A WTW pH meter, respectively). Solutions percolating the microbial mat and the controls were analysed in order to determine the composition of the porewater. Water was collected from the bottom of the tanks, in direct contact with sediments, and filtered at 0.2 µm. Concentrations of F^-, Cl^- and SO_4^{2-} were determined by ion chromatographic analyses. For these analyses, water samples were diluted 90 times with milli-Q water. Concentrations of Ca, Mg, Si, Na, K and B and Sr were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES). For ICP-AES analyses, filtered samples were acid stabilized (with HCl 2%) soon after collection. For these analyses, water samples were diluted with nitric acid at 2% 50 times for B and Sr, 150 times for K and Na and 3200 times for Ca and Mg. Finally, the concentration in Si (measured as H_4SiO_4) was also checked by continuous flow colorimetric analyses. For these analyses, water samples were diluted 10 times with milli-Q water. Commercial mineral water (Volvic) was used as a control. Ionic activities and saturation index were calculated using the Visual Minteq software.

RESULTS

Mineralogical analyses of the sediments

SEM and XRD analyses were combined to identify the major mineral phases forming the sediments beneath the microbial mats (Fig. 6.2A and B, and 6.3, respectively). The detected mineral assemblage was characteristic of an evaporitic environment as this is the case in lake Salada de Chiprana. Gypsum ($CaSO_4 \cdot 2H_2O$) with XRD peaks at 13.5º, 24.1º and 33.9º (2Θ) was the most abundant phase. Quartz (SiO_2) with XRD peaks at 31 and 24.3° (2Θ) and kieserite, an evaporitic hydrated magnesium sulphate ($MgSO_4 \cdot H2O$) with peaks at 30.4º, 31.1º and 21.4º (2Θ) were also detected. The presence of gypsum was confirmed by Raman analyses (Fig. 6.4A) with peaks at 415, 494 and 670 cm-1 attributed to the bending of the SO_4 tetrahedra (v2 symmetric, v2 symmetric and v4 antisymmetric respectively) and peaks at 1008 and 1136 cm-1 resulting from the SO_4 stretching vibrations v1 and v3 (Liu et al., 2009; White, 2009). Aragonite was also evidenced by Raman spectroscopy (Fig. 6.4B) with diagnostic peaks at 155 and 207 cm-1 that correspond to the transitional and librational lattice modes, and 704 and 1085 cm-1 attributed respectively to the bending (v4 vibration) and symmetric stretching (v1 vibration) of the carbonate group (Urmos et al. 1991; White 2009). An additional carbonate mineral phase, likely corresponding to a (Ca,Mg)-calcite, was detected in diffractograms (peaks 34.28º and 46º, Fig. 6.3) but not by Raman spectroscopy. Raman spectro-microscopy and XRD analyses were consistent with analyses performed by SEM-EDXS. Additional phases not evidenced by XRD could be detected in the sediments by SEM suggesting that they are minor and/or poorly crystalline. This includes an abundant Mg- and Si-rich phase (Fig. 6.2E).

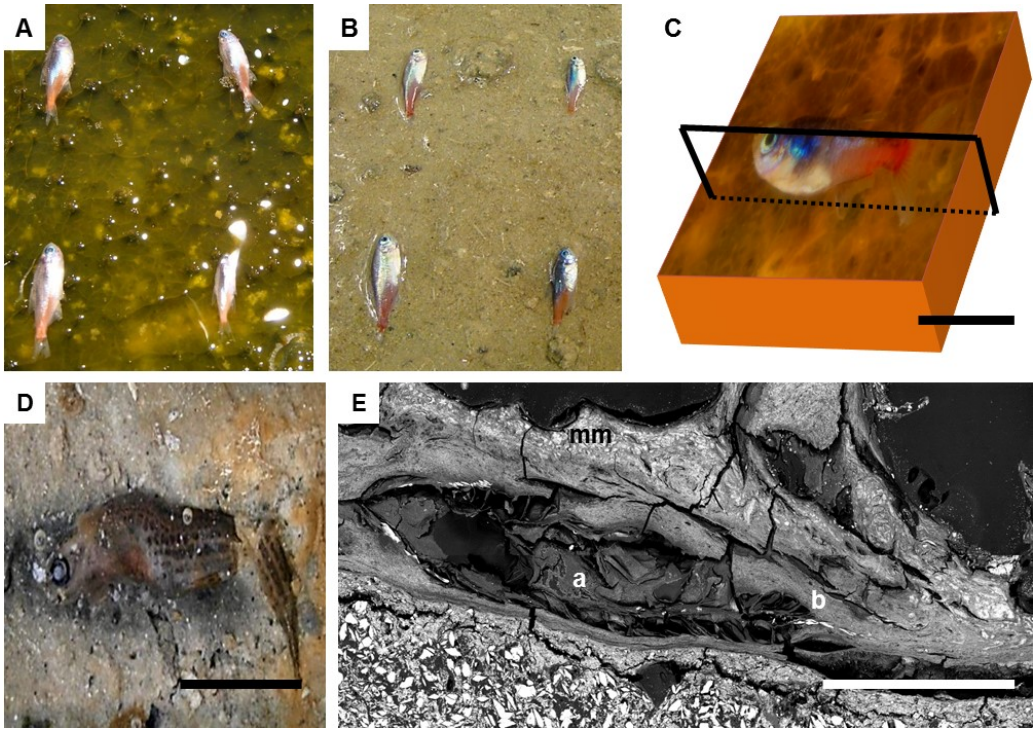

Fig. 6.1: Experimental setup showing how fish were placed (**A**) on the mat and (**B**) directly on the sediments in the control tank at the beginning of the experiment. (**C**) Sketch showing the plane of the section for the SEM preparation. (**D**) SEM image of the fish trapped by the microbial mat after 4 years; (mm) microbial mat; (a) swim bladder; (b) vertebral column. Scale bar represents 1 cm in (C), (D) and (E).

Water composition

Physico-chemical parameters at the beginning of the experiment were: a temperature of 20.8 ± 1.3ºC, a pH of 8.4 ± 0.4 and a conductivity of 52.9 ± 0.6 mS·cm-1 for the microbial mat tank. The control tank, i.e. the one without microbial mat, had a temperature of 20.7 ± 1.4 ºC, a pH of 8.3 ±0.5 and a conductivity of 50.1 ± 1.8 mS·cm-1 (Table 6.1). Solutions percolating the control and the microbial mat tanks contained large amounts of Cl- (between ~660 and 830 mM) and SO42- (between ~847 and 1031 mM) consistently with the evaporitic origin of the sediments (Table 6.1). Concentrations were different between the control and the microbial mat tanks but this could be due to some variations in the mineralogical composition in the different tanks. In addition, the opaque lid covering the control tank likely affects evaporation as suggested by the comparison with the initial values of the water column conductivity (Table 6.1). Differential evaporation may also explain differences observed in Na or Cl for example between the control and the experiments. Mg (between 466 and 546 mM) and Na (576 and 694 mM) were also major elements in water. Ca (>12mM) and K (between 7.8 and 9 mM) were present at lower, yet relatively high concentrations. Several elements were also present at lower concentrations (below 1 mM). Concentrations of Si provided by ICP-AES (0.12 mM) and colorimetric analyses (0.14 mM) were consistent (Table 6.1). All these elements, in particular Mg, Ca and Si likely originated from the interactions between the solution and the sediments underlying the microbial mats. Based on these results and water pH (8.5) and temperature (20ºC), the ionic activities of several

components were calculated. Saturation indexes (SI) of the solutions with several Mg-silicate phases, defined as the decadic logarithms of the ratio of the ion activity product over the solubility product constant (Ks), were calculated. Solubilities (expressed as Log Ks), estimated at 25ºC, of kerolite, sepiolite stevensite and talc were 25.79, 15.76 (Stoessell 1988), 25.45 (Chahi et al. 1997) and 23.02 (Jones 1986) respectively. Solutions in the microbial mat and the control tanks were significantly oversaturated with these different Mg-silicate phases, with SI values of 5.54 for kerolite, 3.52 for sepiolite, 5.71 for stevensite and 8.31 for talc in the microbial mat tank (see Supplementary Tables S6.1 and S6.3 for activities and S6.2 and S6.4 for saturation indexes).

Table 6.1: Concentrations (in mM) of cations and anions in the water percolating the sediments, from the initial water pool, the tank with microbial mat and the control (without mat) tank, determined by ICP-AES, colorimetry and ion chromatography. Measurements of dissolved silica, anions and cations, the uncertainty of the measurements was better than 5%.

		Initial conditions (mM)	Mat tank (mM)	Control tank (mM)
Water Conditions	T (ºC)	20.8	21.8 ± 2.3	21.7 ± 1.9
	pH	8.4	8.5 ± 0.6	8.2 ± 0.4
	Conductivity ($mS \cdot cm^{-1}$)	49.4	54.9 ± 1.7	52.3 ± 2.4
Technique	**Element**			
ICP-AES	B	-	1.68	1.77
	Ca	12.22	12.18	12.41
	K	8.87	9.02	7.85
	Mg	570.43	546.35	466.57
	Na	563.68	693.98	576.46
	Si	-	0.12	0.18
	Sr	-	0.14	0.20
Colorimetric Analysis	H_4SiO_4	-	0.14	0.15
Ion Chromatography	F^-	-	0.55	0.00
	Cl^-	610.36	831.58	663.52

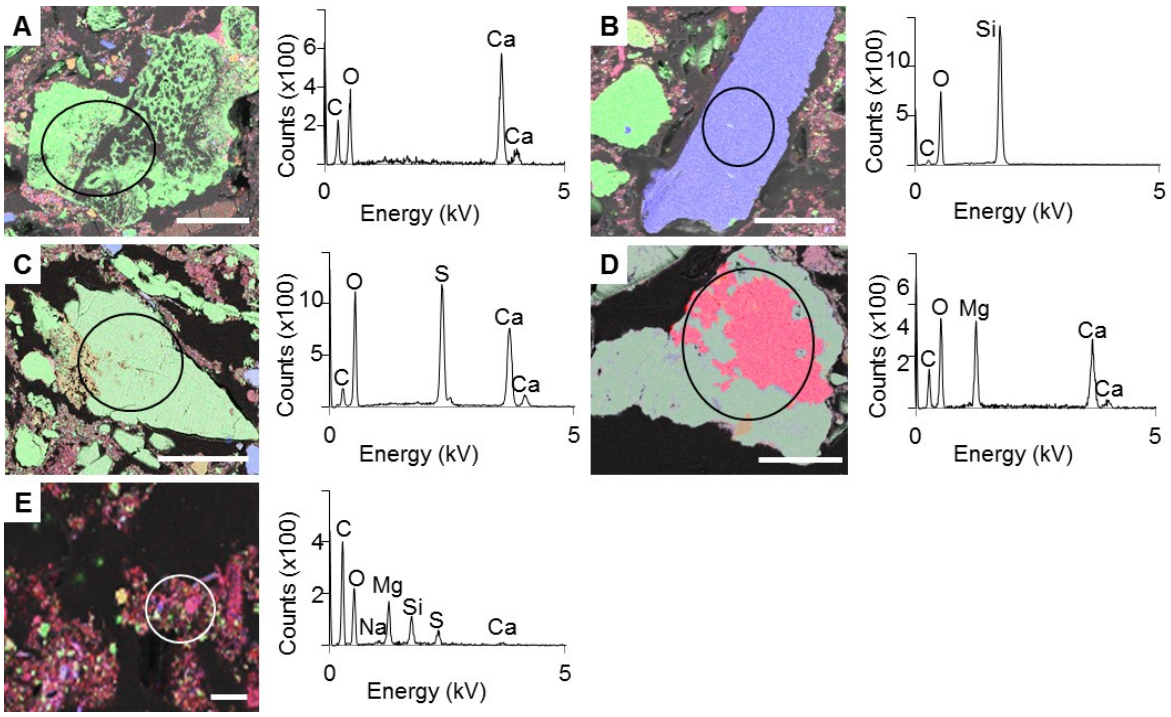

Fig. 6.2: SEM-EDXS maps of the sediment beneath the microbial mat with the corresponding EDXS spectra. (**A–E**) Different mineral phases observed in the sediment. Color legend: green-Ca; blue-Si; red-Mg. Scale bar represents 100 µm for (A–D) and 10 µm for (E).

Electron microscopy analyses of the carcasses

Control fish, as described in previous experiments (Iniesto et al. 2013b) decayed dramatically after a few months. At this time, the collection of the few residues of degraded fish over the sediment was impossible. Soft tissue was completely decayed and bones were disarticulated and broken at day 90 (Fig. 6.1D). Long-term observations (more than a year) when we observed the first precipitates in the experiments could therefore not be performed on controls.

Table 6.2: Elemental composition of the Mg-silicate phase as measured by TEM-EDXS on a FIB foil extracted from the inner tissue of a fish covered by microbial mat after 4 years. Notice that the ratio Mg/Si is close to ¾ (0.77).

Element	Peak position (keV)	Atom%	Error%
F K	0.677	5.14	0.29
Na K	1.041	1.58	1.38
Mg K	1.253	38.63	0.05
Si K	1.739	49.75	0.05
S K	2.307	1.42	1.09
Cl K	2.621	3.48	0.45
Total		100	

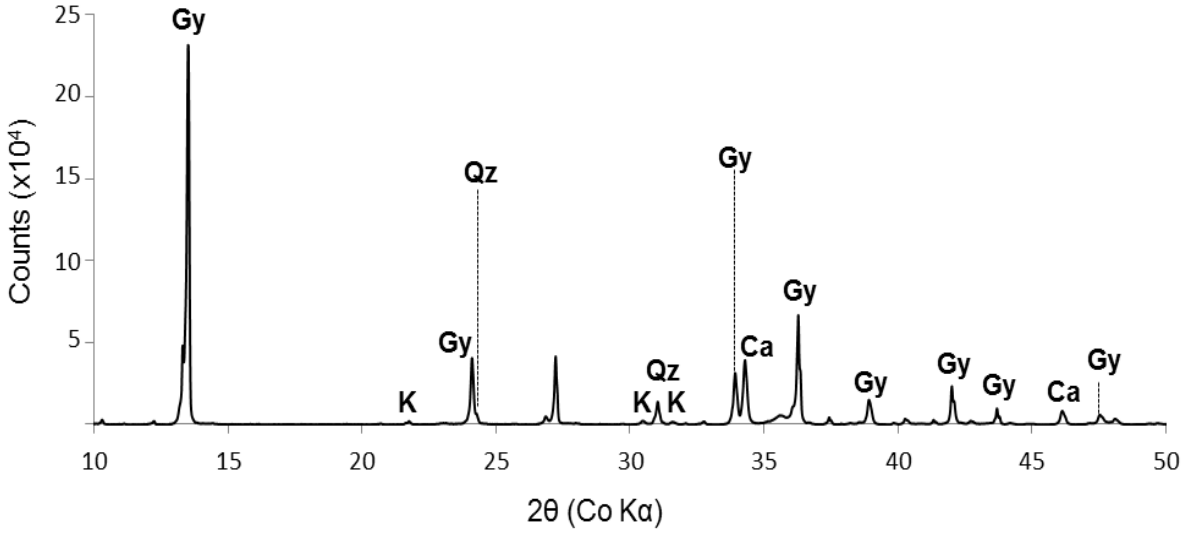

Fig. 6.3: XRD spectrum of the sediment beneath the microbial mat. Gy: gypsum (CaSO4.2H2O); K: kieserite (MgSO4.H2O); Qz: quartz (SiO2); Ca: calcite ((Ca,Mg)CO3) with undetermined Ca/Mg ratio. The diagram shows data from 10 to 50º (2θ).

In contrast, after 4 years of incubation in microbial mats, carcasses inside the mats were exceptionally preserved at the macroscopic scale, as shown notably by the near intact state of the swim bladder (Fig. 6.1E-a) and the vertebral column (Fig. 6.1E-b). The precise delimitation of the interface between the fish and the biofilm was sometimes difficult to determine by SEM in secondary or backscattered electron modes (Fig. 6.5A) because of the limited contrast difference between the biofilm and the external tissues of the fish such as muscles. For this reason, the position of the fish was determined by the detection of bones or fins, easily identified by their high chemical contrast (arrow in Fig. 6.5A) due to the presence of Ca and P. Although the transition between the mat and the fish was unclear, several inner soft-tissues appeared in the SEM as light grey areas (Supplementary Fig. S6.1). SEM observations of these lighter zones showed the presence of a widespread mineral phase rich in Mg- and Si (circle in Fig. 6.5A and 6.5B). This mineral phase was not detected in samples collected after a shorter incubation time as described in Iniesto et al. (2013). In addition, bacillary cells were observed by SEM within the preserved tissues of the fish (arrow, Fig. 6.5C).

Further observations and characterization of this Mg- and Si-rich phase was achieved based on STEM analyses of the FIB foils extracted from the inner tissues of a fish incubated within microbial mats for 4 years (Fig. 6.6; Supplementary Fig S6.1 shows the SEM zones chosen for FIB). Microbial cells embedded in the Mg-Si phase were observed on the FIB foil (arrow, Fig. 6.7). The transversal section of one of these cells appeared as a round shape filled with epoxy, and clearly delimited by an envelope measuring 50-100 nm, also rich in Mg and Si. The surrounding matrix contained nanometer-sized Mg-silicate grains with a homogeneous bright contrast. A Mg/Si ratio of ~0.77 was assessed by EDXS analyses for this phase (Fig. 6.6A-G and Table 6.2), which was poorly crystalline as suggested by SAED patterns (Fig. 6.6H). Minor amounts of Cl and F which may have

precipitated during the sample drying process were also detected. Cl may alternatively be related to the epoxy resin (Fig. 6.6F-G and 6.6I).

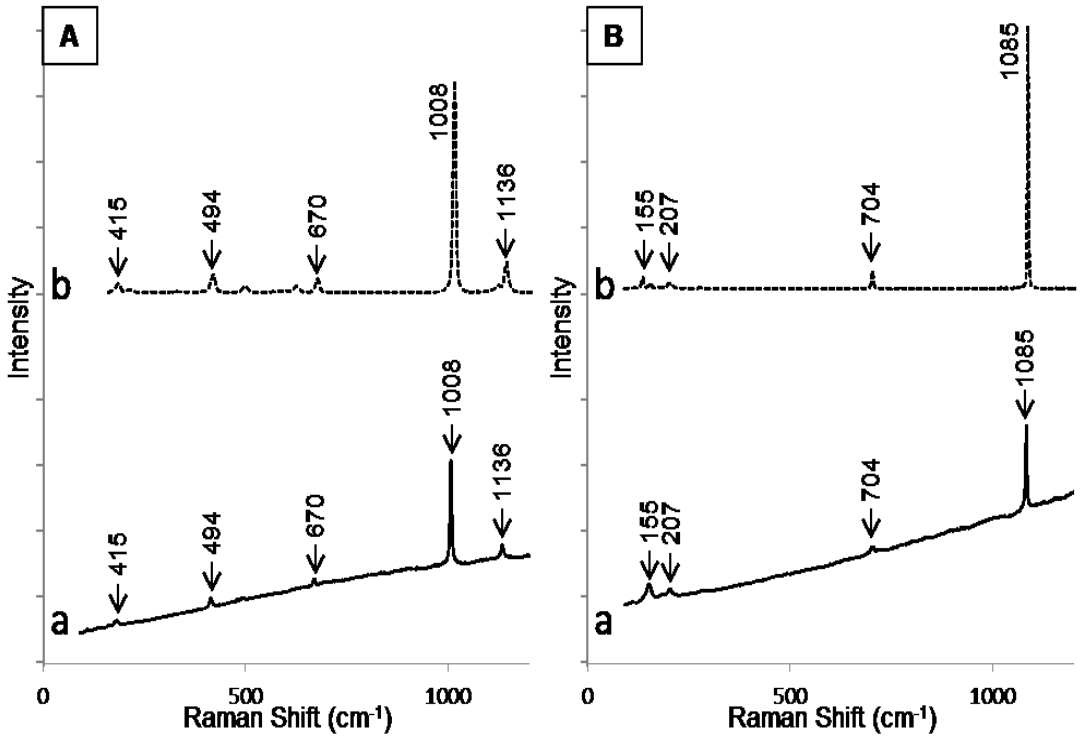

Fig. 6.4: Raman spectra of gypsum (**A**) and aragonite (**B**) as detected in the sediment beneath the microbial mat (a) and corresponding reference spectra from rruff database (b). Bands relevant for identification have been highlighted in both cases.

The Si- and Mg-rich phase was also present in fish carcasses incubated for 5 years. This phase formed a whole mineralized layer in the upper zone of the microbial mat (arrow, Fig. 6.8A). Interestingly, the mineral phase appeared also close to the surface of the body and bones, especially over the dorsal fin: the hemitrichum that composes each thorn of the dorsal fin showed a Mg-silicate coating (arrow, Fig. 6.8B), and several bones seemed to be partially replaced by this phase (Fig. 6.8C-F). In addition, the upper face of the head showed sometimes a similar replacement (Fig 6.9A and 6.9B). Close examination of the upper face of the head of the fish suggested that the replacement of the bone by Mg-silicate was gradual (Fig. 6.9C), from a zone where calcium and phosphorous (principal constituent of bones) were predominant (Fig. 6.9D) to an external zone, rich in Si and Mg (Fig. 6.9F).

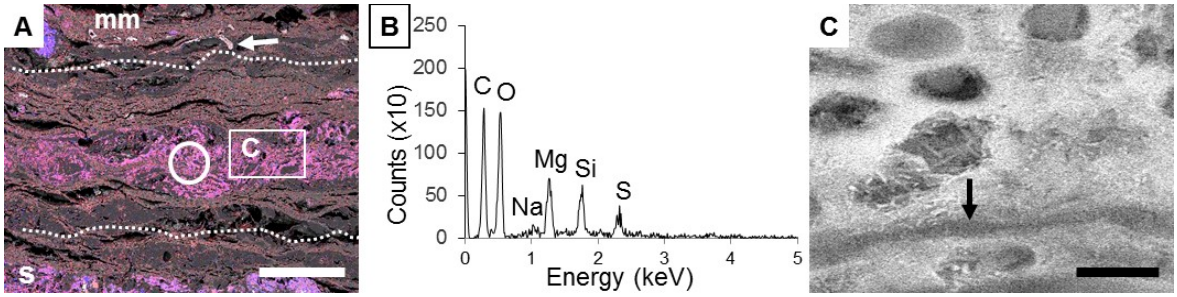

Fig. 6.5: Detection of a Mg silicate phase in the inner tissues of the fish after 4 years of incubation within a microbial mat. (**A**) Composite SEM-EDXS map of the section of the carcass incubated with a microbial mat. Si appears in blue and Mg in red. Dashed lines show the approximate limits of the fish. The circle marks the inner zone of the fish tissues analyzed by EDX in (**B**). Arrow points a fishbone. The box shows the zone highlighted in (**C**). (**B**) EDXS spectrum of the area outlined by a circle in (**A**). (**C**) SEM image of the inner mineralized tissue. Arrow highlights microbial filaments. Scale bar represents 500 µm in (A) and 20 µm in (C).

DISCUSSION

Iniesto et al. (2013b) have demonstrated that microbial mats can favour the preservation of fossils. After the placement of a carcass at the top of the microbial community, the whole system experienced modifications that led to the quick coverage of the body by microorganisms. The microbial coverage generated a protective environment that delayed decay and promoted pseudomorphism allowing the exceptional preservation of carcasses in microbial mats (Guerrero et al. in press). In addition, the surfaces of bodies were copied as impressions in the inner face of the sarcophagi (Iniesto et al. 2013a).

The present work focuses on the possible additional role played by mineral precipitation in this preservation process. Our initial hypothesis was that increased preservation of fish tissues within the microbial mats may have resulted from enhanced mineral precipitation by microorganisms, forming some kind of mineral crust around the fish and therefore protecting them from further degradation. No such crust was observed here around carcasses but, in contrast, we observed a gradual transition from the microbial mats to the fish tissues at the micrometer scale. However, we detected the formation of an authigenic poorly-crystalline Mg-silicate phase in relation with the decay of carcasses in the presence of a microbial mat in a long-term (few years) experiment.

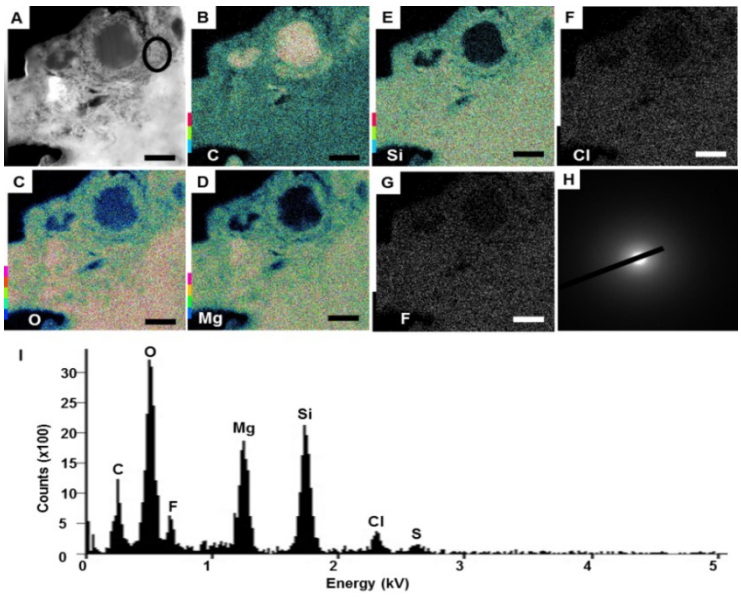

Fig. 6.6: TEM-EDXS maps of the inner fish tissue after 4 years. (**A**) TEM image of the analyzed area. (**B-G**) Carbon, oxygen, magnesium, silicon, chlorine and fluor maps. (**H**) SAED pattern obtained on the Mg-silicate phase. The presence of diffuse rings only indicates the poor crystallinity of the phase. (**I**) EDXS spectrum of the area outlined by a circle in (**A**). All scale bars represent 1μm.

A similar poorly crystalline and authigenic Mg-rich silicate phase has been observed previously in microbialites and lithifying mats (e.g.: Kempe et al. 1991; Arp et al. 2003; Benzerara et al. 2010). In addition, the occurrence of similar silicates has been reported repeatedly in other diverse environments such as hot springs (Jones and Peng 2014) or marine sediments (Badaut and Risacher 1983). Stevensite, sepiolite or kerolite (an hydrated talc-like phase) have been proposed as possible authigenic candidate phases forming under these conditions (e.g., Tosca and Masterson 2014). Tosca and Masterson (2014) showed that these phases can be distinguished based on FTIR spectroscopy and/or thermogravimetric analyses (TGA). However, these bulk techniques were not applicable in our study, where we needed to discriminate micrometer-scale phases lying within the fish carcasses from abundant phases within the sediments. Yet, the ~3/4 ratio for Mg/Si provided by EDXS analyses pointed to kerolite and/or stevensite as the most likely Mg-silicate phases forming within fish. Interestingly, it has been noted by former studies that these phases can delicately fossilize microbial structures after cell death (Souza-Egipsy et al. 2005). Consistently with some of the previous studies, our data showed the embedment of microbial cells in this kerolite-like phase, supporting the hypothesis of its relatively good fossilization potential. Not only the inner tissue seems to be templated finely by this kerolite-like phase, but a gradual replacement by Mg-silicates of the hydroxyapatite forming the bones of the fish was also observed. It is important to notice that the kerolite-like phase was only described in long-term experiments. Control fish without microbial mats decayed dramatically after only two months (Iniesto et al. 2013b) and this phase was not observed in any of them.

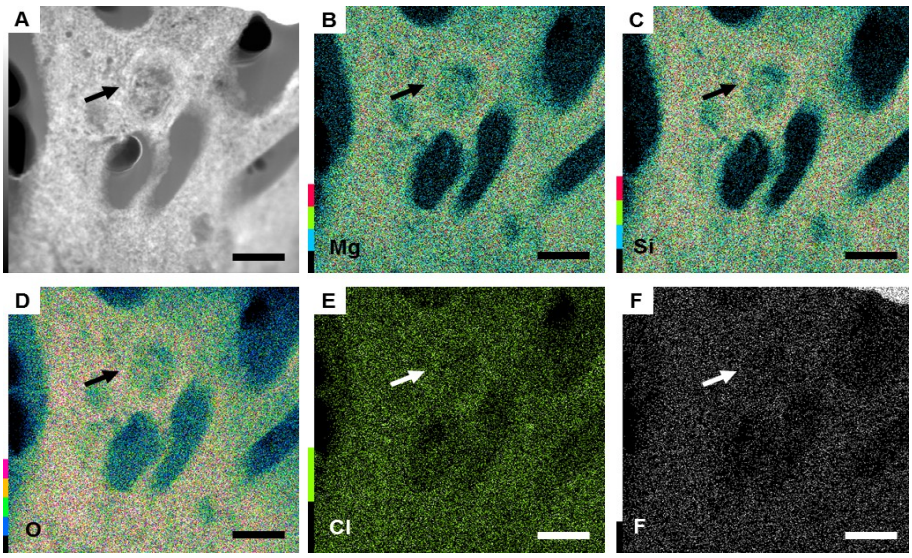

Fig. 6.7: Bacteria present in the preserved 4-years fish tissue, embedded in a Mg-rich silicate matrix. (**A**) TEM image of the section of the bacteria. (**B-F**) magnesium, silicon, oxygen, chlorine and fluor maps highlighting the enrichment in Si and Mg of the matrix. All scale bars represent 1µm.

The mechanisms leading to the precipitation of this phase can be discussed. Mg and Si were provided by the solution which dissolved the Mg and Si-rich phases contained

within the sediments. Quartz was the only Si-containing phase detected by DRX but is an improbable source of Si considering its low solubility. Poorly crystalline Mg-silicate phases, observed by SEM-EDX but not detected by DRX, may provide an alternative source of Si (Fig. 6.2E). In that case, this kind of phase may dissolve in the sediments but reprecipitate in the fish due to a local microenvironment favourable to precipitation possibly in connection with the activity of microorganisms (see below). Another possibility is that the frustules of dead diatoms provide a source of Si for Mg-silicate precipitation. It will be important in future studies to better quantify the budgets of these different Si reservoirs. Mineralogical and sedimentological observations have documented similar early diagenetic hydrated Mg-silicate mineral phases in carbonate-dominated deposits (Friedman 1965; Noack et al. 1989; Calvo et al. 1999). Such phases can precipitate at ambient temperature in supersaturated solutions (e.g., Gac et al. 1977; Kent and Kastner 1985; Tosca et al. 2011) following the reaction (written for kerolite):

$$3\ Mg^{2+} + 4\ SiO_2\ (aq) + 5\ H_2O \rightarrow Mg_3Si_4O_{10}(0H)_2.H_2O + 6\ H^+ \quad (1)$$

As shown by reaction (1), precipitation of kerolite is favoured by increased activities of Mg^{2+}, SiO_2 and a higher pH. Moreover, it is known that kinetic barriers can limit precipitation at low temperatures and that precipitation of such a mineral phase may therefore only occur when the solution is significantly oversaturated. According to chemical analyses, solutions were significantly oversaturated (>5 for kerolite or stevensite) in the present experiments and therefore these phases may have precipited everywhere in the tanks. The preferential precipitation of Mg-silicate at discrete sites within the fish may have resulted from a local pH increase during the transformation of carcasses within microbial mats. During the coverage of bodies by the microbial mat, a short-standing phase with an increase of acidity in the system was actually described, although a stable basic pH (above 8.5) was recovered afterwards, affecting especially the top-face of the fish (Iniesto et al. 2015). This increase with an unequal spatial distribution depends dramatically on the presence of these microbial communities. This relatively high pH can be explained by the photosynthetic activity in the upper oxic layers (Jonkers et al. 2003), as well as, locally, the dissolution of hydroxyapatite of bones. The preferential localization of Mg-silicate precipitation around bones and fines in the upper face of carcasses may therefore result from these latter processes. Moreover, the presence of preferential nucleation surfaces may counteract kinetic barriers and favour precipitation (e.g., De Yoreo and Velikov 2003). It may be hypothesized that microbial cell surfaces and/or extracellular polymers, decaying tissues and/or bone surfaces may provide such preferential nucleation sites for Mg-silicates, similarly to what was described by Ueshima and Tazaki (2001) for Fe-rich silicate precipitation. Depending on the stability of this phase, tissues and bones would be preserved as a talc-like fossil. Tosca and Masterson (2014) mentioned that this kind of poorly crystalline phases should be replaced by more crystalline phases over geological times, through dehydration and concomitant crystallographic ordering. This may decrease slightly the quality of structural preservation at the submicrometer-scale. However, it should be noted that some ancient fossils of soft tissues of metazoans have been preserved by clays, including within metamorphic rocks of relatively high grade

(Zhang and Briggs 2007; Galvez et al. 2012; Wacey et al. 2014), supporting the plausibility of fossil preservation by such silicates.

In conclusion, the present experiments show the first steps of early mineralization of fish carcasses covered by microbial mats. They confirm the influence of microbial mats on the fossilization of metazoans. More than 4 years after the beginning of the decay, inner tissues or bones were gradually replaced by a fine and poorly-crystalline Mg-silicate phase. Fluids permeating the system were oversaturated with kerolite and stevensite, as a likely result of partial dissolution of the underlying sediments rich in quartz, gypsum, kieserite, aragonite other minor Mg- and Si-containing phases and/or the frustules of dead diatoms composed of amorphous silica. Moreover, local processes, possibly linked with biological activity (rising pH and/or offering preferential nucleation sites) may have played as well in favouring the precipitation of authigenic Mg-silicates. While exceptional preservation can be the result of different abiotic processes such as obrution and/or stagnation (Seilacher et al. 1985), the present results evidence that precipitation of authigenic silicates may favour this preservation at least in some environments, here characterized by alkaline and Mg-rich fluids. The experimental fossilization of a vertebrate by a talc-like mineral is here revealed for the first time and further work on the long-term fate of such fossils replaced by this type of mineral phase will be needed.

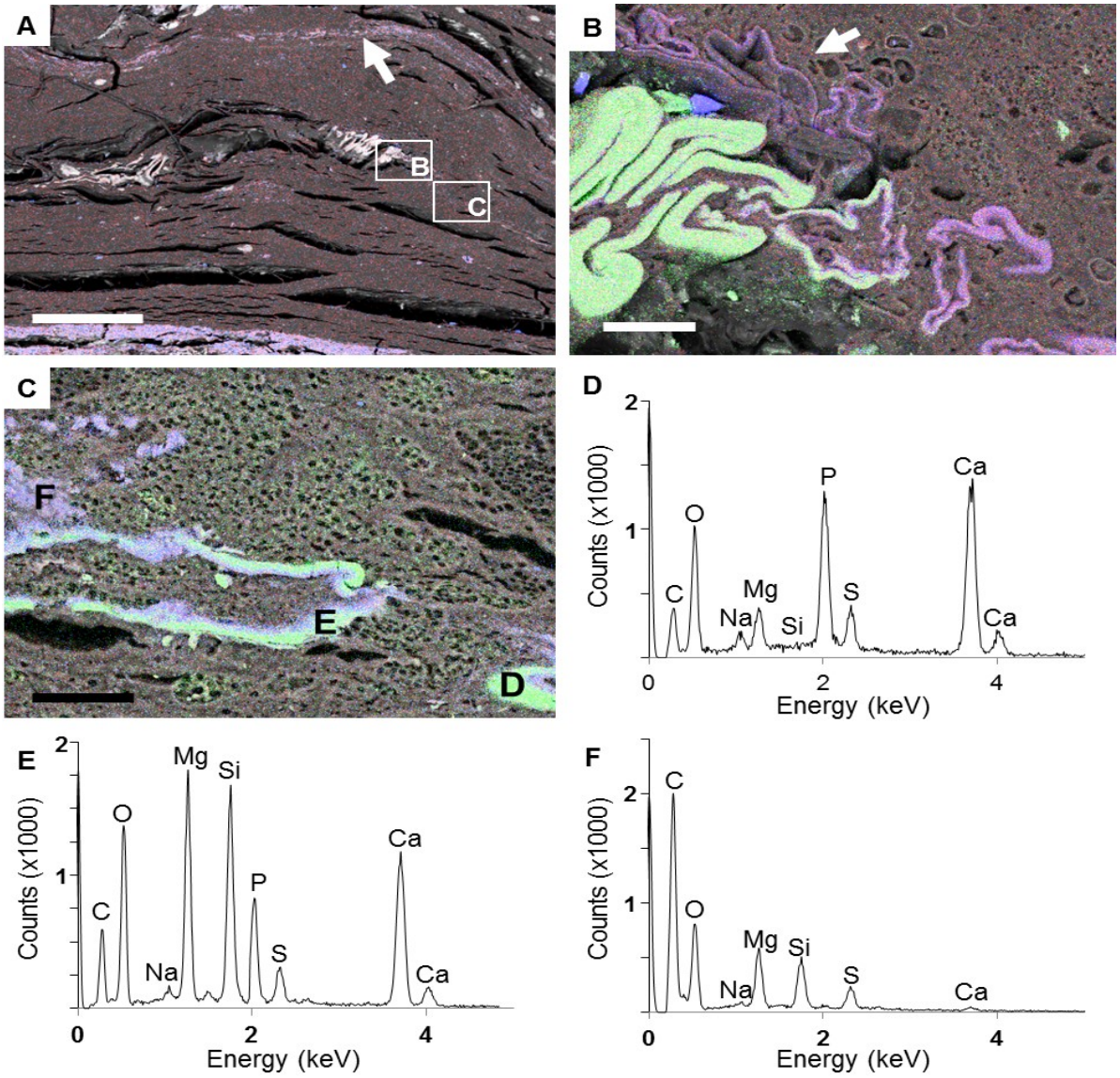

Fig. 6.8: SEM analyses of a fish after 5 years of incubation within a microbial mat. (**A**) General view of the section with a Si-Mg enriched layer in the top of the mat (arrow). The rectangle shows the zone of the preserved fish magnified in (**B**). (**B**) EDXS maps of Ca (green), Si (blue) and Mg (red) in the region of the dorsal fin (in green). A precipitated layer of Mg-silicate is highlighted by the arrow. (**C**) End of the dorsal fin where replacement of the bone (made by Ca –green- and P) by the Mg-silicate phase (purple) is observed. (**D-E-F**) EDXS spectra corresponding to the spots labelled in **C**. Scale bars represent 500 µm in (A) and 30 µm in (B) and (C).

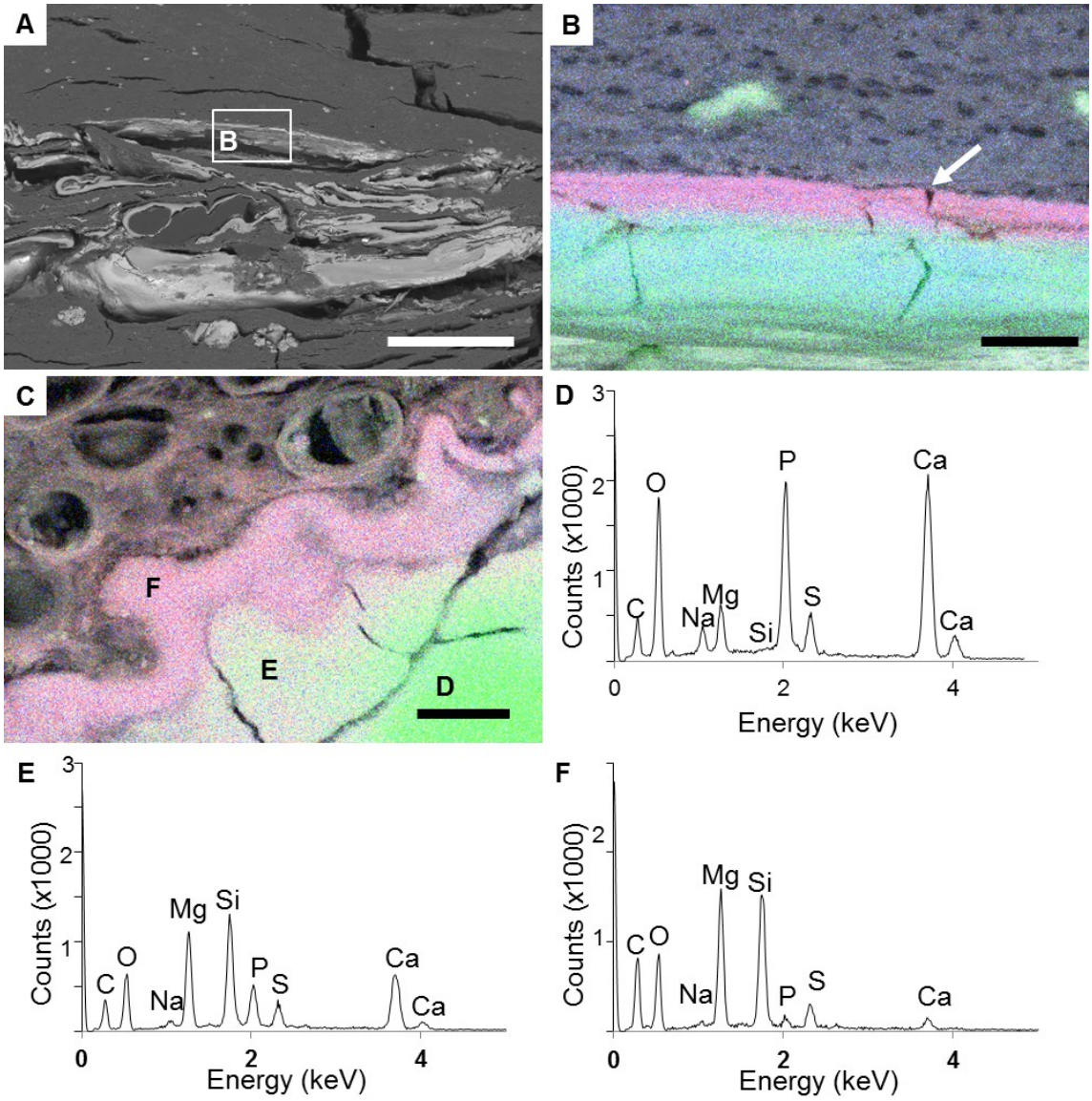

Fig. 6.9: SEM analyses of the head of a fish carcass after 5 years of incubation in a microbial mat. (**A**) General view of the head. (**B**) EDXS maps showing the localization of Ca (green), Si (blue) and Mg (red) around the head bone (green) as outlined in (**A**). The Mg-silicate phase (purple) delineates continuously the external side of the skull bone in the upper face. (**C**) Area of the skull where the bone is replaced by the Mg-silicate phase. (**D-E-F**) EDXS spectra corresponding to the spots shown in (**C**). Scale bars represent 400 µm in (A) and 10 µm in (B) and (C).

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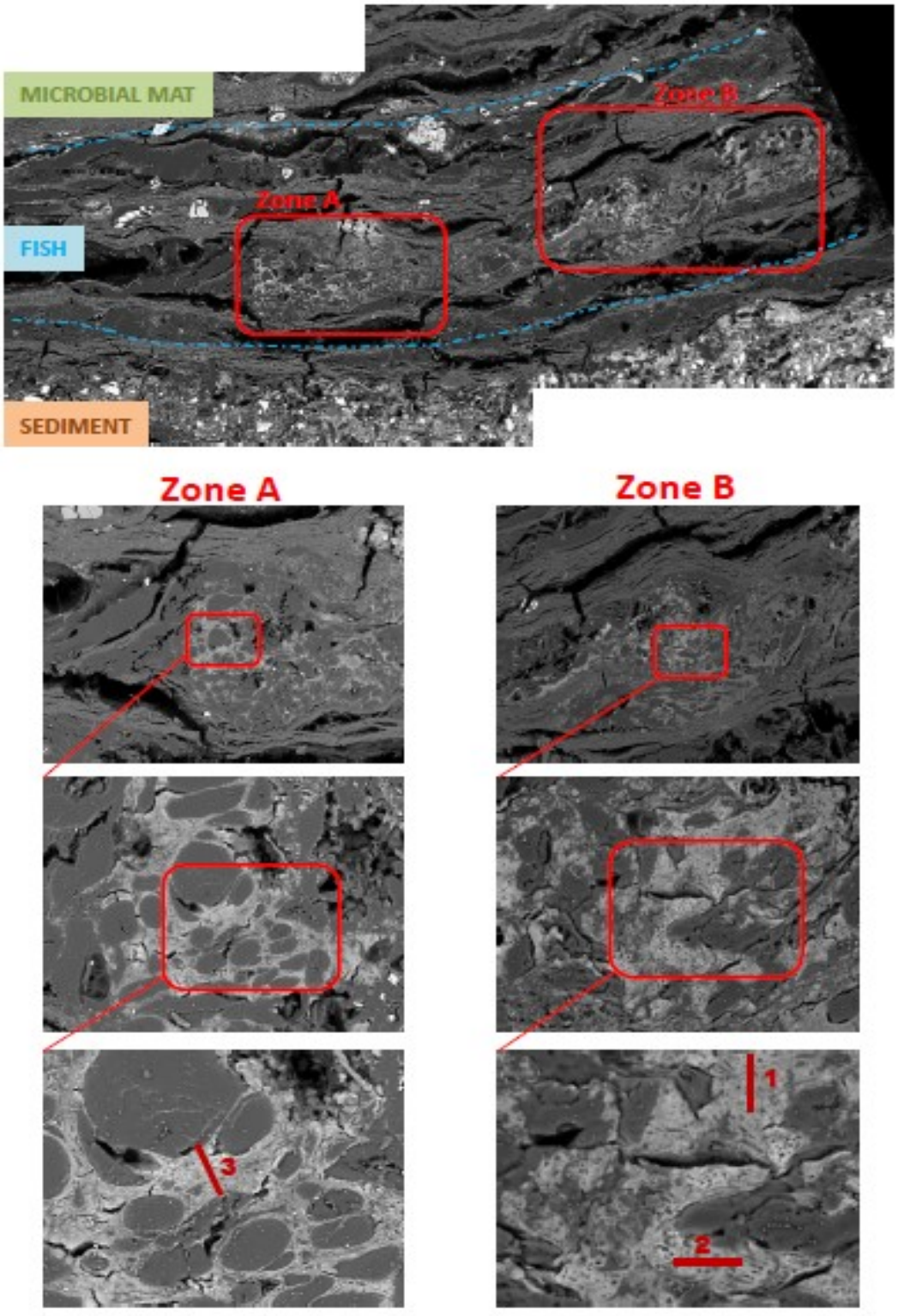
SUPPLEMENTARY FIG S6.1



SUPPLEMENTARY TABLE S6.1

Supplementary Table S6.1: Microbial mat activities.

	Concentration mmol/l	Activity	Log activity
$BF(OH)^{3-}$	1,4041E-20	1,1561E-20	-19,937
$BF2(OH)^{2-}$	1,1066E-37	9,1119E-38	-37,04
$BF3OHF^{-}$	3,807E-57	3,1347E-57	-56,504
BF^{4-}	1,3554E-75	1,116E-75	-74,952
Br^{1-}	0,00072872	0,00060003	-3,222
Ca^{2+}	0,0013678	0,00062872	-3,202
$CaCl^{+}$	0,00082124	0,00067621	-3,17
CaF^{+}	2,2178E-19	1,8262E-19	-18,738
$CaH_2BO_3^{+}$	9,3814E-06	7,7246E-06	-5,112
$CaOH^{+}$	3,1209E-08	2,5697E-08	-7,59
$CaSO_4$ (aq)	0,0099827	0,013101	-1,883
Cl^{-}	0,53452	0,44013	-0,356
F^{-}	2,8139E-17	2,3169E-17	-16,635
H^{+}	3,8405E-09	3,1623E-09	-8,5
$H_{10}(BO_3)_4^{2-}$	4,3235E-10	1,9874E-10	-9,702
$H_2BO_3^{-}$	0,00026821	0,00022085	-3,656
$H_2SiO_4^{2-}$	1,3234E-10	6,0832E-11	-10,216
H_3BO_3	0,001005	0,0013189	-2,88
$H_3SiO_4^{-}$	6,8004E-06	5,5994E-06	-5,252
H_4SiO_4	0,00012111	0,00015894	-3,799
$H_4SiO_4SO_4^{2-}$	9,3948E-06	4,3185E-06	-5,365
$H_5(BO_3)_2^{-}$	3,1169E-07	2,5665E-07	-6,591
$H_8(BO_3)_3^{-}$	3,5579E-08	2,9296E-08	-7,533
HF (aq)	7,7111E-23	1,012E-22	-21,995
HF_2^{-}	1,1021E-38	9,0748E-39	-38,042
HNO_2 (aq)	2,8026E-11	3,6781E-11	-10,434
HSO_4^{-}	3,1234E-08	2,5718E-08	-7,59
K^{+}	0,0051243	0,0042194	-2,375
KCl (aq)	0,00072898	0,0009567	-3,019
KF (aq)	3,4049E-20	4,4685E-20	-19,35
KOH (aq)	1,2118E-08	1,5903E-08	-7,799
KSO_4^{-}	0,0031708	0,0026109	-2,583
Mg^{2+}	0,069995	0,032174	-1,492
$MgCl^{+}$	0,066608	0,054845	-1,261
MgF^{+}	6,5761E-17	5,4148E-17	-16,266
$MgH_2BO_3^{+}$	0,00029735	0,00024484	-3,611
$MgOH^{+}$	0,000030366	0,000025003	-4,602
$MgSO_4$ (aq)	0,40944	0,53734	-0,27
Na^{+}	0,41613	0,34264	-0,465
NaCl (aq)	0,06085	0,079859	-1,098
NaF (aq)	5,8323E-18	7,6542E-18	-17,116

NaH_2BO_3 (aq)	0,000099311	0,00013033	-3,885
NaOH (aq)	6,9353E-07	9,1017E-07	-6,041
$NaSO_4^-$	0,21692	0,17862	-0,748
NO_2^-	0,00001	8,2342E-06	-5,084
OH^-	2,6341E-06	2,1689E-06	-5,664
SiF_6^{2-}	1,2926E-107	5,9418E-108	-107,226
SO_4^{2-}	0,20779	0,095514	-1,02
Sr^{2+}	0,000017705	8,1382E-06	-5,089
$SrCl^+$	6,3766E-06	5,2505E-06	-5,28
SrF^+	7,4478E-22	6,1325E-22	-21,212
$SrH_2BO_3^+$	7,4876E-08	6,1653E-08	-7,21
$SrOH^+$	1,3684E-10	1,1267E-10	-9,948
$SrSO_4$ (aq)	0,00014679	-3,833	

SUPPLEMENTARY TABLE S6.2

Supplementary Table S6.2: Microbial mat saturation indexes.

Mineral	log IAP	Sat. index
Brucite	15,508	-1,933
Chalcedony	-3,799	-0,19
Cristobalite	-3,799	-0,389
Epsomite	-2,512	-0,351
Gypsum	-4,221	0,392
Halite	-0,822	-2,361
KCl(s)	-2,731	-3,631
kerolite	31,327	5,54
Lime	13,798	-19,48
$Mg(OH)_2$ (active)	15,508	-3,286
$Mg_2(OH)_3Cl{:}4H_2O$(s)	22,159	-3,841
MgF_2(s)	-34,763	-26,677
Portlandite	13,798	-9,29
Quartz	-3,799	0,268
Sepiolite	19,619	3,518
Sepiolite (A)	19,619	0,839
SiO_2 (am,gel)	-3,799	-1,047
SiO_2 (am,ppt)	-3,799	-1,013
stevensite	31,157	5,707
talc	31,327	8,307

SUPPLEMENTARY TABLE S6.3

Supplementary Table S6.3: Control tank activities.

	Concentration mmol/l	Activity	Log activity
$BF(OH)^{3-}$	1,5071E-07	1,3103E-07	-6,883
$BF2(OH)^{2-}$	1,0722E-11	9,3217E-12	-11,031
$BF3OHF^{-}$	3,3296E-18	2,8947E-18	-17,538
BF^{4-}	1,07E-23	9,3027E-24	-23,031
Br^{1-}	0,00077957	0,00067775	-3,169
Ca^{2+}	0,00062042	0,00035444	-3,45
$CaCl^{+}$	0,00063466	0,00055177	-3,258
CaF^{+}	1,0689E-06	9,2929E-07	-6,032
$CaH_2BO_3^{+}$	6,2889E-06	5,4675E-06	-5,262
$CaOH^{+}$	1,6663E-08	1,4487E-08	-7,839
$CaSO_4$ (aq)	0,011147	0,01542	-1,812
Cl^{-}	0,73275	0,63704	-0,196
F^{-}	0,00024056	0,00020914	-3,68
H^{+}	3,6374E-09	3,1623E-09	-8,5
$H_{10}(BO_3)_4^{2-}$	8,6444E-10	4,9384E-10	-9,306
$H_2BO_3^{-}$	0,00031894	0,00027728	-3,557
$H_2SiO_4^{2-}$	1,1361E-10	6,4902E-11	-10,188
H_3BO_3	0,001197	0,0016559	-2,781
$H_3SiO_4^{-}$	6,8716E-06	5,9741E-06	-5,224
H_4SiO_4	0,00012258	0,00016958	-3,771
$H_4SiO_4SO_4^{2-}$	0,000016839	9,6197E-06	-5,017
$H_5(BO_3)_2^{-}$	4,6535E-07	4,0457E-07	-6,393
$H_8(BO_3)_3^{-}$	6,6692E-08	5,7981E-08	-7,237
HF (aq)	6,6034E-10	9,1349E-10	-9,039
HF_2^{-}	8,5052E-13	7,3943E-13	-12,131
HNO_2 (aq)	4,1265E-11	5,7084E-11	-10,243
HSO_4^{-}	6,1763E-08	5,3696E-08	-7,27
K^{+}	0,0031424	0,002732	-2,564
KCl (aq)	0,00064813	0,0008966	-3,047
KF (aq)	1,8879E-07	2,6117E-07	-6,583
KOH (aq)	7,4435E-09	1,0297E-08	-7,987
KSO_4^{-}	0,0040598	0,0035295	-2,452
Mg^{2+}	0,027383	0,015644	-1,806
$MgCl^{+}$	0,044396	0,038597	-1,413
MgF^{+}	0,00027335	0,00023765	-3,624
$MgH_2BO_3^{+}$	0,00017192	0,00014946	-3,825
$MgOH^{+}$	0,000013983	0,000012157	-4,915
$MgSO_4$ (aq)	0,39431	0,54548	-0,263
Na^{+}	0,25054	0,21781	-0,662
NaCl (aq)	0,053116	0,073478	-1,134

NaF (aq)	0,000031749	0,000043921	-4,357
NaH_2BO_3 (aq)	0,000075196	0,00010402	-3,983
NaOH (aq)	4,1825E-07	5,7859E-07	-6,238
$NaSO_4^-$	0,27268	0,23706	-0,625
NO_2^-	0,000014699	0,000012779	-4,893
OH^-	2,4947E-06	2,1689E-06	-5,664
SiF_6^{2-}	6,003E-30	3,4294E-30	-29,465
SO_4^{2-}	0,34908	0,19942	-0,7
Sr^{2+}	0,000011617	6,6367E-06	-5,178
$SrCl^+$	7,1286E-06	6,1975E-06	-5,208
SrF^+	5,1925E-09	4,5143E-09	-8,345
$SrH_2BO_3^+$	7,2609E-08	6,3125E-08	-7,2
$SrOH^+$	1,0569E-10	9,1886E-11	-10,037
$SrSO_4$ (aq)	0,00018067	0,00024993	-3,602

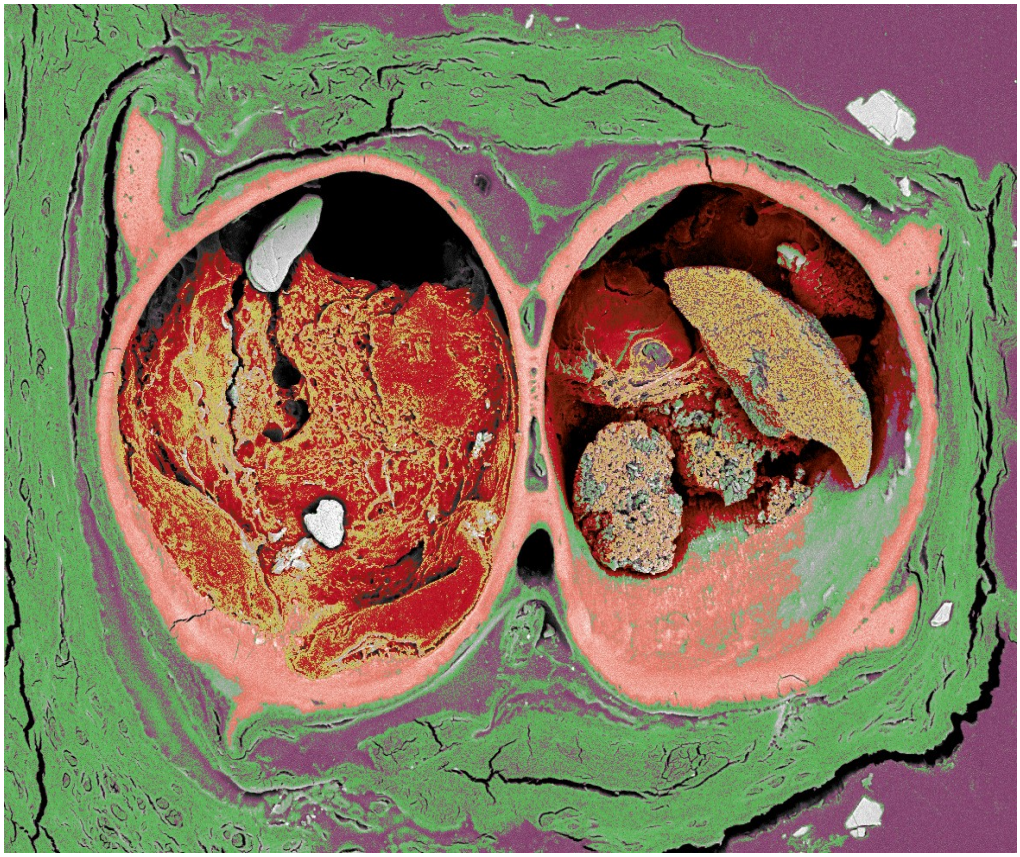
SUPPLEMENTARY TABLE S6.4

Supplementary Table S6.1: Control saturation indexes.

Mineral	log IAP	Sat. index
Brucite	15,194	-2,246
Chalcedony	-3,771	-0,162
Cristobalite	-3,771	-0,361
Epsomite	-2,506	-0,345
Gypsum	-4,151	0,462
Halite	-0,858	-2,397
KCl(s)	-2,759	-3,659
kerolite	30,5	4,713
Lime	13,55	-19,729
$Mg(OH)_2$ (active)	15,194	-3,6
$Mg_2(OH)_3Cl{:}4H_2O$(s)	21,693	-4,307
MgF_2(s)	-9,165	-1,079
Portlandite	13,55	-9,539
Quartz	-3,771	0,296
Sepiolite	19,077	2,976
Sepiolite (A)	19,077	0,297
SiO_2 (am,gel)	-3,771	-1,019
SiO_2 (am,ppt)	-3,771	-0,985
stevensite	30,336	4,886
talc	30,5	7,48

CAPÍTULO 7

DISCUSIÓN GENERAL



INFLUENCIA DE LOS TAPETES MICROBIANOS EN LA PRESERVACIÓN

La mediación de los microorganismos en múltiples aspectos del procesos de fosilización está ampliamente aceptada en la actualidad, gracias principalmente a las inferencias derivadas del estudio del registro fósil (*e.g*. Gall 1990, 2001; Briggs 2003a, 2003b). Tratando de explicar las fases sucesivas que dan lugar a la formación de un fósil, una serie de aproximaciones experimentales han puesto de relieve la capacidad de las poblaciones microbianas de modificar los ambientes en el entorno de un cadáver, creando así condiciones físico-químicas que favorecen su preservación y posterior mineralización (Sagemann et al. 1999, Wilby et al. 1996). Estos experimentos, generalmente llevados a cabo con microorganismos heterótrofos que formaban biofilms simples durante la descomposición de los cuerpos, permitieron describir el efecto diferencial de este proceso sobre los distintos tejidos de un organismo, teniendo algunos de ellos un potencial mayor de preservación, e.g. la musculatura bajo el exoesqueleto de un crustáceo (Briggs y Kear 1993, Hof y Briggs 1997) o el sistema digestivo en artrópodos (Butler et al. 2015). Además, se pudo determinar que, en presencia de este tipo de microorganismos, la descomposición de los especímenes era rápida (Briggs 2003b) y se liberaban ácidos orgánicos (Ben-Yaakov 1973). Todo ello generaba un ambiente favorable para la fosfatización de los tejidos (Briggs y Kear 1994, Hof y Briggs 1997, Sagemann et al. 1999, Wilby et al. 1996). Este microambiente se ve favorecido en los organismos con exoesqueleto, el cual constituye una barrera frente al medio externo, quedando el interior aislado (Briggs y Wilby 1996). Además de músculos y otros tejidos, la fosfatización mediada por la descomposición microbiana habría participado en la fosilización de huevos de crustáceo (Briggs y Kear 1993), explicando así la preservación de los fósiles encontrados en yacimientos de la formación Doushantuo (Neoproterozoico, China) (Xiao y Knoll 1999). Sin embargo, la existencia de un ambiente ácido fruto de la descomposición microbiana no siempre desemboca en la fosfatización de los restos, pudiendo quedar preservados también en carbonato cálcico (Martin et al. 2003), por lo que los mecanismos que determinan la fase mineral durante el proceso de preservación son aún poco conocidos. Otros trabajos han logrado replicar estructuras blandas como embriones (Raff et al. 2008), llevando a cabo una caracterización de las poblaciones y grupos que podrían estar implicados en su degradación temprana (Raff et al. 2013). La anterior serie de experimentos han permitido establecer un relato lógico de cómo podría ser la fosilización mediada por microorganismos. Este esquema asume que la preservación excepcional y la mineralización autigénica de las etapas tempranas de la formación del fósil debían transcurrir en anoxia y a pH ácido (Sagemann et al. 1999, Wilby et al. 1996). Dichas condiciones constituirían una protección frente a los depredadores (la anoxia crearía un ambiente hostil de difícil acceso) y, teóricamente, favorecería la precipitación de diversos minerales tales como fosfato cálcico (Briggs y Kear 1993, 1994) o sulfuros ferrosos (Brock et al. 2006). Sin embargo, los resultados obtenidos en los ensayos tafonómicos realizados con tapetes microbianos no siempre se han ajustado a las condiciones propuestas en este esquema.

En nuestros experimentos con tapetes se observa que la llegada de un cadáver a su superficie supone una perturbación de la comunidad, generada por la entrada de materia orgánica alóctona. En esta etapa inicial el cadáver sufre una descomposición relativamente rápida, que tiene como resultado una pérdida notable de volumen (ver capítulos 2, 3 y 4). A pesar de ello, la presencia del tapete bajo el cadáver implica un notable retraso en la descomposición, modificando la secuencia del proceso con respecto al deterioro de los cadáveres en ausencia de este tipo de comunidad microbiana. Al comparar los grados de descomposición en ranas y peces en función de la presencia/ausencia de tapete, se puede determinar el retraso en el proceso de forma semicuantitativa, atendiendo a criterios arbitrarios. De acuerdo a los resultados obtenidos en los experimentos, un cadáver sobre sedimento al cabo de una semana presenta un grado de descomposición comparable al de un cuerpo que lleve 15 días sobre tapete microbiano en el caso de las ranas (capítulo 3) o hasta 30 días en el caso de los peces (capítulo 2). Además, estas diferencias tienden a acentuarse a partir de la segunda semana de experimentación, como muestra la comparación de la pérdida de volumen de los cadáveres en base a las medidas de longitud, grosor y anchura en peces (Fig. 2.4) y ranas (Fig. Suplementaria S3.5), coincidiendo con el final de la fase de distensión provocada por la actividad de la microbiota interna de los cuerpos (Cambra-Moo et al. 2008). Los controles en ausencia de tapete, tanto de peces como de ranas, sufren una descomposición significativamente mayor como muestran los dendrogramas de las figuras 2.3 y 3.9. Por otro lado, durante los primeros días, las capas superiores del tapete microbiano comienzan a atrapar progresivamente los cuerpos que han sido depositados en su superficie. Este crecimiento activo de las cianobacterias estaría asociado con la descomposición inicial anteriormente descrita ya que, durante la degradación de los tejidos del cadáver por la actividad microbiana heterótrofa y por autolisis, se liberarían compuestos orgánicos de fácil asimilación así como nutrientes inorgánicos, los cuales servirían de sustrato para el rápido desarrollo de las cianobacterias. Debido a la activación local de su crecimiento, comienza a quedar impreso en el tapete el contorno y relieve de los cuerpos, proceso que continúa hasta la formación y consolidación de un sarcófago como se discutirá posteriormente.

Los primeros días que se suceden tras la deposición del cuerpo sobre el tapete microbiano, en los que la descomposición es relativamente rápida, serían los que más se asemejarían al modelo planteado en base a los experimentos realizados con biofilms simples (e.g. Briggs y Kear 1993, 1994; Hof y Briggs 1997, Sagemann et al. 1999). En lo que se refiere al microentorno en contacto directo con los cadáveres de peces, el análisis con microelectrodos (capítulo 5) registra de forma temprana (durante la primera semana) importantes cambios en la concentración de oxígeno disuelto (DO) y el valor del pH, aunque estas variaciones aparecen desacopladas en el tiempo (Fig. 5.4). En un primer momento se registra un descenso del DO como resultado de la descomposición rápida por parte de microorganismos heterótrofos aerobios, valores que se mantienen así durante varios días, aunque el pH permanece básico. En este recién generado ambiente anóxico los microorganismos heterótrofos no pueden respirar de forma aerobia y los metabolismos alternativos para la oxidación de materia orgánica, como la fermentación y/o la respiración anaerobia de diferentes sustratos, continuarían la

descomposición. Como resultado de estos nuevos procesos catabólicos se liberan, además de los nutrientes inorgánicos anteriormente mencionados, diversos ácidos orgánicos e inorgánicos, que explicarían la reducción del pH observada a los 7 días en el interior del cadáver. Sin embargo, este efecto parece tener un carácter local ya que en el exterior del cuerpo el pH se mantiene en valores cercanos a 8 (Fig. 5.4). El pH más elevado en el exterior del cadáver podría explicar la presencia de minerales en forma de gránulos o de una fina cubierta, posiblemente de carbonatos, en la superficie de algunos peces (Fig. 2.5), proceso de precipitación que se vería dificultado en el interior del cuerpo debido a los valores ácidos de pH. Durante esta primera etapa, mientras que los ejemplares depositados sobre el tapete microbiano presentaron un grado de preservación elevado, los controles se descompusieron de forma significativa. Sin embargo, las condiciones de pH ácido y anoxia fueron más pronunciadas en los tanques con sedimento (controles) que en los que contenían los tapetes microbianos, por lo que la generación de estas condiciones no parece ser suficiente para explicar las diferencias en la preservación. Por otra parte, pasados los primeros días de descomposición moderadamente activa, tanto el interior del pez como su entorno recuperan valores elevados de DO y pH. La comunidad microbiana es responsable de este cambio en el microambiente debido a la fotosíntesis oxigénica llevada a cabo por las cianobacterias del sarcófago. Por lo tanto, el modelo propuesto y aceptado, desarrollado con biofilms heterótrofos, presenta algunos aspectos que no concuerdan con las observaciones obtenidas en los ensayos con tapetes microbianos. En consecuencia, es necesario replantear el modelo establecido para las etapas tempranas de fosilización, al menos, en los casos que impliquen tapetes microbianos basados en fotosíntesis. Nuestros resultados, sin embargo, sí serían coherentes con experimentos previos que tuvieron en cuenta la acción de poblaciones fotosintéticas oxigénicas en los que se observó que, durante la descomposición de insectos, se podían desarrollar ciertos velos verdes (fotosintéticos) que favorecían el mantenimiento de la articulación de los cadáveres (Peñalver-Mollá 2002). En este contexto, la experimentación en tafonomía con tapetes microbianos jugaría un papel clave en el establecimiento de nuevas hipótesis.

La primera etapa, en la que el cuerpo está expuesto, es de corta duración. Rápidamente, entre 15 y 30 días en el caso de cadáveres de pequeño tamaño, la capa superior del tapete que había fijado el cuerpo a la superficie forma una cubierta completa sobre el cadáver, generando un sarcófago. El tiempo que tarda en formarse el sarcófago varía en función del tamaño del cuerpo y del estado de activación de la comunidad microbiana (ver capítulos 2 a 6). Esta envuelta, que actúa como barrera entre el resto orgánico y el exterior, es un elemento esencial para explicar la fosilización mediada por tapetes microbianos. Una vez dentro del sarcófago, el cadáver se encuentra protegido de forma mecánica frente a la desarticulación. Está descrito que la abrasión producida por las corrientes de agua existentes en los fondos son responsables de la desconexión y desarticulación de los cadáveres (Martín-Abad 2015) y, por lo tanto, de la fragmentación de los restos. La cubierta microbiana, debido a las propiedades inherentes del tapete (e.g. compactación, existencia de una red entrelazada de filamentos gruesos de cianobacterias, EPS), impediría la rotura de los cuerpos y favorecería la integridad de los restos fósiles. El análisis mediante RMI de peces a los 8

meses, ranas a los 2 y 6 meses y moscas a los 12 meses (Fig. 7.1) muestra, a través del sarcófago, la completa articulación de los cadáveres. Además, el mantenimiento de la integridad del cuerpo se ve acompañado por la preservación de los órganos internos. La observación mediante SEM permitió detectar en peces a los 24 meses tejidos de sostén o la vejiga natatoria, tejido adiposo o músculo en ranas, u órganos como el aparato digestivo y los ovarios en moscas a los 66 meses (5.5 años). En el caso de las ranas el aplanamiento fue mayor que en los otros tipos de animales, por lo que la RMI no mostró resultados satisfactorios debido a la falta de volumen y la consecuente dificultad para obtener planos válidos. Aun así, al realizar un corte transversal en ranas de 3 años se pudo observar la existencia, no sólo de una completa articulación, sino también de tejidos blandos como piel o músculos (capítulo 3).

Fruto de la íntima interacción entre el cuerpo y el sarcófago y del crecimiento progresivo del tapete, en la capa interna de la envuelta se genera un molde en negativo del cadáver dando lugar a una impresión de gran fidelidad (capitulo 4). Como se ha explicado anteriormente, el tapete recubre rápidamente el cuerpo. La cubierta está compuesta por células microbianas y por una densa matriz de EPS que le da soporte y cohesión a la creciente comunidad. El molde, que no sólo mantiene la forma y contorno del animal, sino que es capaz de copiar incluso estructuras de pequeño tamaño como las vellosidades del ala de una mosca, de unos 3-4 µm (capítulo 4), se detecta en muestras de más de 5 años, por lo que su capacidad potencial de preservación es grande. Además, nuestras observaciones mostraron que las impresiones generadas en el sarcófago estaban formadas principalmente por células de pequeño tamaño embebidas en la matriz de EPS, mientras que los filamentos de mayor tamaño quedaban en una posición más alejada respecto del cadáver dando consistencia al sarcófago. Por otro lado, las estructuras externas del cuerpo, como los ojos, no sólo quedan marcadas en forma de impresión en la capa microbiana, sino que aparecen cubiertas por multitud de estas pequeñas células. Estos microcuerpos de forma bacilar han sido interpretados por algunos autores como melanosomas (Lindgren et al. 2012), i.e., orgánulos celulares encargados de contener la melanina producida por los melanocitos, y que serían responsables de la coloración observada en ciertos fósiles (Vinther et al. 2008). Sin embargo, la diferenciación entre microorganismos y melanosomas está actualmente sujeta a una fuerte controversia (Moyer et al. 2014). De acuerdo a los criterios propuestos por Moyer et al. (2014) consideramos que las estructuras observadas en nuestro caso se corresponderían con bacterias, según su patrón de distribución y su densidad. Por otro lado, mediante la obtención de nutrientes a partir de la descomposición de los tejidos más fácilmente biodegradables, los microorganismos son capaces de crecer sobre el cuerpo, manteniendo y sustituyendo las estructuras que les sirven de sustrato, imitando por ejemplo las diferentes capas del ojo y generando su réplica (capítulo 4).

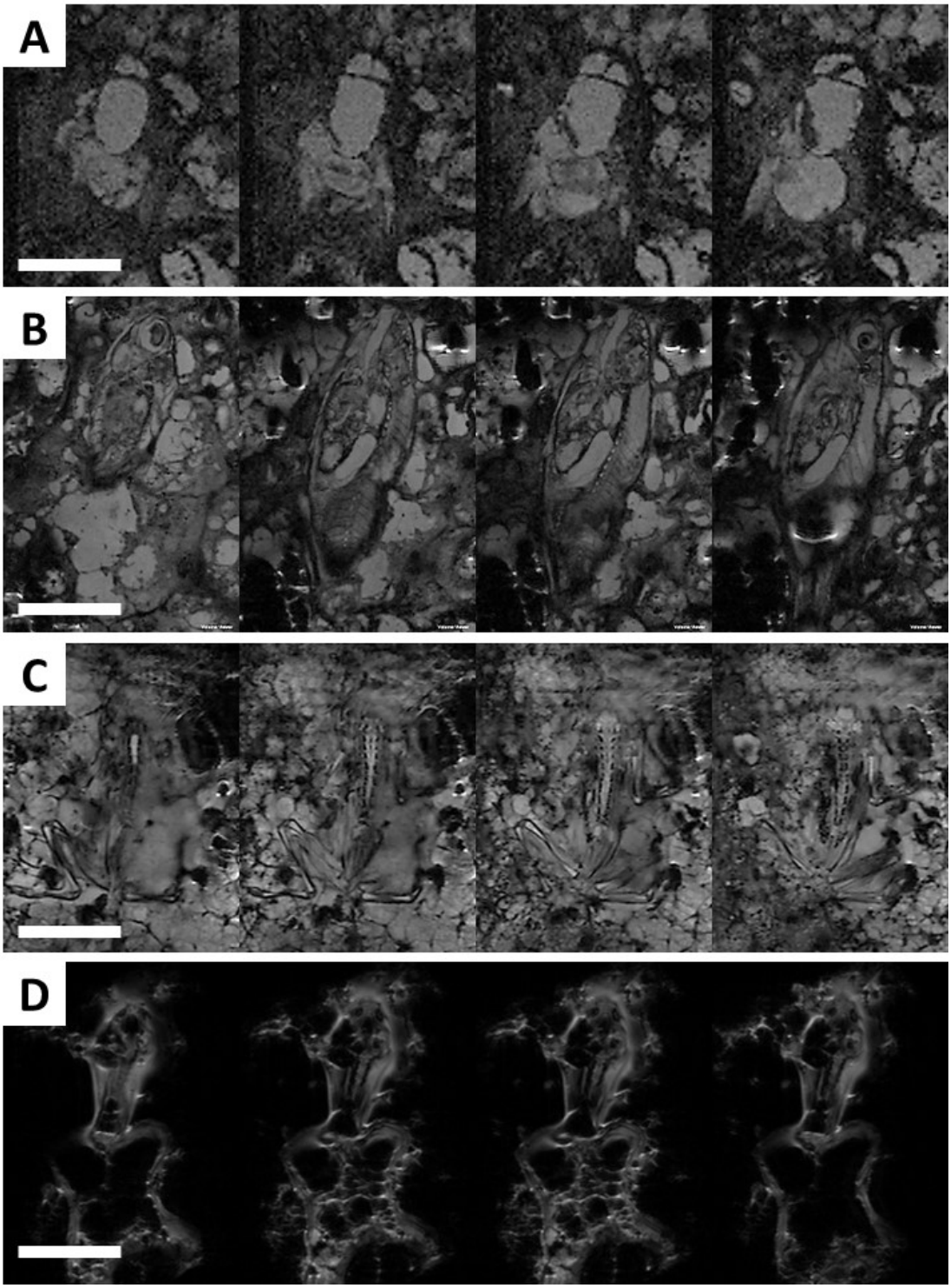


Fig. 7.1: Imágenes de resonancia magnética mostrando una secuencia de planos que revelan el grado de preservación en el interior del sarcófago de: (**A**) *Musca domestica* (12 meses en el tapete), (**B**) *Paracheirodon innessi* (8 meses en tapete). (**C**) y (**D**): *Hymenochirus boettgeri* después de 2 y 6 meses en el interior del sarcófago, respectivamente. Escalas: A: 0.5 cm; B-D: 1 cm.

Otro aspecto que se ve afectado por el aislamiento creado por la cubierta y la modificación de los factores físico-químicos que afectan al cadáver es la formación de distintos precipitados. El microambiente generado es de vital importancia para poder comprender y describir las posibles fases minerales que se originen durante la mineralización de los cuerpos, algo que, salvo en el caso de la preservación orgánica, es necesario para la formación final de los fósiles. Así, en el ambiente óxico y básico, se observó en muestras a tiempos largos la aparición de un silicato rico en magnesio, tanto en el interior de los cuerpos como en la interfase tapete-cadáver. Este mineral, que presentaba un bajo grado de cristalización, tenía una composición similar a la del talco (capítulo 6). A los cuatro años el mineral se encontraba en el interior de los cuerpos, rodeando las células de los microorganismos que habían colonizado los órganos. A los cinco años el mineral recubrió de forma externa la cara superior de algunas zonas del cadáver, y en ciertos puntos parecía incluso haber sustituido gradualmente algunos huesos como en el caso de las aletas del pez. La aparición diferencial de la cubierta mineral rica en Si y Mg podría estar relacionada también con el microambiente registrado por los microelectrodos, óxico y básico en la zona superior y anóxico y con un pH algo más bajo en la base, y se explicaría por el progresivo enterramiento del cuerpo en la comunidad microbiana. A medida que el tapete crece, el cadáver va quedando cada vez más cerca de las capas profundas y, por lo tanto, se encuentra expuesto en su cara inferior a procesos metabólicos diferentes a los observados en las capas superiores. Así, a la altura de la capa roja el pH se reduce y el DO decae hasta la anoxia, impidiendo la formación del silicato de magnesio en esta zona del cadáver.

En resumen, los experimentos que se presentan en este trabajo muestran que tras la deposición de un resto orgánico en la superficie de un tapete se forma un sarcófago microbiano lo que conlleva una serie de cambios ambientales directamente relacionados con las características inherentes del propio tapete. El cadáver, al sufrir un cubrimiento activo, ve reducido drásticamente el periodo de exposición. Una vez aislado, el cuerpo es sometido a unas condiciones físico-químicas diferentes a las del exterior que favorecen su conservación mediante tres mecanismos distintos: la preservación orgánica, la mineralización mediada por microorganismos y la formación de moldes. Aunque estos tres mecanismos han sido previamente descritos (Briggs 2003a), hasta ahora no existía un modelo que reuniese los tres procesos.

PRESERVACIÓN ORGÁNICA EN TAPETES MICROBIANOS

Los experimentos realizados ponen de relieve el excepcional potencial de preservación de los tapetes microbianos y su papel en dos de los procesos conocidos que permiten la formación de fósiles de tejido blando: la preservación orgánica y la mineralización autigénica. La preservación orgánica, i.e. el mantenimiento de los tejidos orgánicos originales sin producirse sustitución mineral, queda favorecida mediante la conservación de los tejidos durante largos periodos de tiempo sin un grado de descomposición elevado (capítulo 1). Aunque la duración de nuestros experimentos es de 5.5 años como máximo, el alto grado de integridad, la baja degradación y el ambiente protector que supone el tapete microbiano hace altamente probable que esta preservación se sostenga en el tiempo. Además, aunque la fosilización es un proceso que puede alargarse durante periodos medidos a escala geológica, un gran número de cambios se producen en las etapas tempranas (Sansom 2014) por lo que su retraso o inhibición a lo largo de estos primeros años puede tener una influencia drástica en el resultado final. Aunque *a priori* podría parecer que este tipo de conservación orgánica es improbable, y en el pasado fue ciertamente controvertida (para más información de la polémica surgida y las sucesivas respuestas de Mary H. Schweitzer y su equipo, ver Asara y Schweitzer 2008, Asara et al. 2007, Buckley et al. 2008), cada vez más trabajos parecen confirmar la influencia del retraso en la descomposición en la formación de fósiles (e.g. Asara et al. 2007, McNamara et al. 2006, 2009; Schweitzer & Horner 1999, Schweitzer et al. 2005). Como es lógico, no todos los tejidos tienen el mismo potencial para ser preservados. Existen tejidos recalcitrantes, como las cutículas de ciertos exoesqueletos de artrópodos, que al ser difícilmente degradables tienen mayor tendencia a perdurar (Butterfield 1990), por lo que existen autores que no dudan en separarlos del resto de tejidos blandos (Briggs 1995). Otros tejidos como la sangre o la médula ósea, sin embargo, serían más sensibles a la descomposición (Custer 1974) por lo que su preservación se ha considerado como altamente improbable. Aunque su aparición se ha interpretado frecuentemente como un artefacto (Martill y Unwin 1997), recientemente Bertazzo et al. (2015) han demostrado, mediante el uso de diferentes técnicas analíticas (e.g. SEM, FIB, TEM, ToF SIMs), la existencia de eritrocitos en restos fósiles de dinosaurio de 75 millones de años, pese a que los huesos analizados no mostraban externamente ninguna señal de preservación excepcional. Esto ha reforzado la idea de una infraestimación del mecanismo de preservación orgánica en el registro fósil. Lo observado en nuestros experimentos, donde secciones de huesos de ranas con una antigüedad de un año mostraban aún médula ósea sin descomponer e incluso sin evidencias de colonización bacteriana (capítulo 4), sería una forma de conservación mediada por tapetes microbianos que podría desembocar en la preservación de estos tejidos lábiles. De hecho, el grado de preservación de los huesos permite la observación de restos de lo que pudiera ser tejido mesenquimático, en el que se encuentran formas que por aspecto y tamaño podrían corresponderse con células pluripotenciales de la línea hematopoyética, dentro de huesos de anuros después de 1.5 años en el interior del sarcófago.

Una posible explicación para la preservación orgánica sería la existencia de una barrera física que actuara como envuelta del organismo (McNamara et al. 2006). En ocasiones, la naturaleza de esta cubierta puede ser endógena (como el hueso que recubre la médula), pero también puede darse el caso de que el cadáver se recubra mediante un elemento ectógeno. Los ejemplares preservados en ámbar, por ejemplo, han sido una fuente importante de información. De hecho, el resto de ADN más longevo que ha sido analizado proviene de un ejemplar fosilizado de termita del Mioceno, con unos 25 millones de años, si bien la validez de esta datación es controvertida (Gutierrez & Marin 1998). Aunque este tipo de inclusiones en ámbar no son frecuentes, existen ciertos yacimientos que han aportado una valiosa información taxonómica gracias a los detalles que muestran los restos preservados, como en el caso de Utrillas (Cretácico Inferior, España). La resina, que posee propiedades antimicrobianas (Martínez-Delclòs et al. 2004), proporciona un entorno en el que la descomposición queda inhibida y, por lo tanto, favorece la preservación de los tejidos no biomineralizados y el mantenimiento de la estructura 3D de los ejemplares, debido a la formación de una cubierta endurecida alrededor de los cuerpos. En el caso de los tapetes microbianos, la preservación se vería igualmente favorecida por un proceso de protección. La formación del sarcófago actúa también a dos niveles una vez que se forma la barrera completa y el cadáver queda aislado: por un lado, la descomposición se ralentiza; y por el otro, el cuerpo se encuentra incluido en un bloque que lo protege y mantiene su integridad física, su articulación y su estructura tridimensional, aunque en el caso de cuerpos blandos éstos tienden a perder grosor con el tiempo. En el caso de organismos con exocutícula, como los insectos, se observó también una reducción del grosor a tiempos largos debido a la creciente potencia de lámina que lo cubre, aunque sin fracturar el cuerpo. Esto podría explicarse por el progresivo reblandecimiento de la exocutícula en el que las partes con un grado mayor de esclerotización, como por ejemplo el tórax, presentan un menor nivel de aplastamiento (Peñalver-Mollá 2002).

MINERALIZACIÓN AUTIGÉNICA EN TAPETES MICROBIANOS

Los experimentos presentados a lo largo de este trabajo han aportado también evidencias de la participación de los tapetes microbianos en la mineralización mediada por microorganismos. Este proceso ha sido frecuentemente presentado como opuesto a la preservación orgánica y se presuponía que debía ocurrir de forma rápida, poco después de la muerte (Sansom 2014). Dicha premisa se basaba en la estrecha relación, demostrada en ensayos de laboratorio, que parecía existir entre la descomposición de los cadáveres y la precipitación de minerales como fosfatos (e.g. Briggs y Kear 1993 o Sagemann et al. 1999). Experimentalmente se había comprobado que la preservación de los tejidos, en el caso de la mineralización autigénica, requería que, fruto de la rápida descomposición de los cuerpos, se generaran gradientes geoquímicos en el entorno de los cadáveres diferentes de los que había al inicio (Hof y Briggs 1997, Sagemann et al. 1999). Sin embargo, como se ha explicado anteriormente, muchos de estos experimentos se llevaron a cabo con biofilms simples, generalmente de carácter heterótrofo. Por un lado, la escasa diversidad funcional de estas comunidades limitaba el número de procesos en los que podrían verse implicadas y, por otro, la presencia mayoritaria de poblaciones heterótrofas determinaría la rápida descomposición de los cuerpos. En este contexto, sí parece necesaria una mineralización rápida, con el propósito de limitar el deterioro que pudiera generarse durante la degradación de los restos. Pero, ¿qué pasaría en un ambiente cuya comunidad microbiana no se viera predispuesta a descomponer de forma rápida el cadáver? En nuestro trabajo se han empleado tapetes microbianos, equivalentes a los que en el pasado pudieron tapizar la mayoría de los fondos de lagos someros y zonas costeras del mundo. Estas comunidades se comportan de forma autónoma ya que están compuestas en parte por poblaciones autótrofas, constituyendo un ecosistema microbiano. Los experimentos realizados han demostrado que, más allá de una primera etapa de corta duración, la descomposición de los cadáveres es limitada y la comunidad tiende a rodearlos y aislarlos en lugar de utilizarlos como fuente de nutrientes. En este marco, la mineralización mediada por microorganismos no vería reducido el espacio temporal en el que puede producirse. De hecho, como se ha dicho anteriormente, en el experimento más largo con peces algunos tejidos blandos internos y huesos estaban siendo remplazados a los 5 años por una fase mineral rica en sílice y magnesio (capítulo 6). Estos tejidos mineralizados podrían preservarse a muy largo plazo manteniendo la forma inicial del cuerpo. No obstante, los cambios ambientales que previsiblemente se darían en condiciones naturales a una escala temporal geológica podrían permitir la precipitación y/o sustitución mineral. Es decir, la aparición en nuestros experimentos de este silicato de magnesio que podría estar actuando como fase mineral de preservación, no descarta la posibilidad de que, bajo diferentes condiciones, esta protección se realice mediante otras fases minerales. En el caso concreto de nuestros sistemas de experimentación (tanques), la conjunción de un pH básico con los equilibrios químicos observados en el agua, junto con un sedimento que mostró una fase poco cristalizada y probablemente reactiva rica en Si y Mg serían la base consecuente para la precipitación del silicato de magnesio observado

en los cadáveres. Hay que destacar que este tipo de fases son conocidas por su capacidad para precipitar de forma espontánea a temperatura ambiente en soluciones sobresaturadas en ambos elementos (e.g., Gac et al. 1977, Kent y Kastner 1985, Tosca et al. 2011). Por otra parte, en los experimentos realizados con ranas (capítulo 3), se ha observado también que parte del encéfalo de ejemplares de 1.5 y 3 años había mineralizado en carbonato cálcico (Fig. 3.8), aunque las condiciones ambientales son comparables a las de los experimentos con peces. Por lo tanto, es probable que la fase mineral en la que se preserven las estructuras blandas no dependa exclusivamente del ambiente y de la composición química, sino que el propio tejido pueda jugar algún tipo de papel en la precipitación diferencial de minerales.

La precipitación está íntimamente relacionada con la composición del agua y las condiciones físico-químicas de la misma. Por lo tanto, aunque en nuestros experimentos los tejidos se han preservado en forma de silicatos y de carbonatos, en un sistema en el que la química y/o la actividad biológica favoreciese la sobresaturación de otros elementos se podría producir la precipitación en el interior del sarcófago de otras fases minerales, teniendo en cuenta las condiciones físico-químicas previamente descritas (i.e., ambiente óxico y básico). En el registro fósil existe un número relativamente elevado de ejemplares que presentan tejidos blandos fosfatizados (e.g. Briggs et al. 2005 o Martill 1988, 1990) y, además, en algunos experimentos tafonómicos llevados a cabo con velos heterótrofos se ha observado fosfatización de tejidos (e.g. Briggs y Kear 1993 o Hof y Briggs 1997) por lo que se ha asumido que se trata de un mecanismo relativamente frecuente de preservación que, sin embargo, en nuestros experimentos parece no producirse. Aunque el motivo principal de su ausencia pudiera ser el pH, puesto que su precipitación ha sido comúnmente asociada a ambientes ácidos (Briggs y Wilby 1996), estudios de mineralogía y química demuestran que su formación también es posible en un ambiente básico (hasta un pH de 11), siempre y cuando la solución se encuentre saturada en fosfatos (Recillas et al. 2012, Song et al. 2002). De hecho, aunque en la reacción de precipitación de la hidroxiapatita (ecuación E1) (Cosmidis et al. 2015) se produce la liberación de protones, si en el ambiente hay suficientes aniones hidroxilo, ambos reaccionarían desplazando el equilibrio hacia la derecha favoreciendo la precipitación mineral:

$$(E1)\ 5Ca^{2+} + 3HPO_4^{2-} + H2O \Leftrightarrow Ca_5(PO_4)_3OH + \mathbf{4H^+}$$

Por lo tanto, la explicación de la ausencia de fosfatización debe provenir, más que del pH ambiental, de algún otro factor. Cabe destacar que en el caso de los experimentos con velos heterótrofos, los cuerpos se descomponían rápidamente, por lo que se consideraba que la degradación era un agente de preservación ya que producía la acidez necesaria para la precipitación de fosfato cálcico (Briggs 2003b). Sin embargo, esta precipitación es probable que no se deba, como se ha dicho antes, al bajo pH sino al incremento localizado de iones fosfato en el entorno cercano al cuerpo en

descomposición, siendo la precipitación de la hidroxiapatita la causa de la disminución del pH, como se deduce de la ecuación E1 (Cosmidis et al. 2015). La actividad biológica libera ortofosfatos mediante la hidrólisis de moléculas orgánicas como proteínas o fosfolípidos (Paytan y McLaughlin 2007), lo que saturaría la solución y, tras reaccionar con el calcio del ambiente, favorecería la precipitación de fosfato cálcico (Cosmidis et al. 2015). En el caso de los tapetes microbianos, puesto que la descomposición es más lenta y menos activa, la liberación de ortofosfatos sería menor, lo que impediría la precipitación de fosfatos en los tejidos.

FORMACIÓN DE MOLDES EN TAPETES MICROBIANOS: UNA TERCERA VÍA DE PRESERVACIÓN

La implicación de los tapetes microbianos en el mantenimiento de la forma de los cadáveres sirve de modelo para explicar uno de los posibles procesos de preservación de la fauna de Ediacara (e.g. Gehling 1999, Steiner y Reitner 2001 o Narbonne 2005). Como ya se ha comentado, existe un trabajo con tapetes microbianos (Darroch et al. 2012) que muestra cómo se forma en ellos una impresión del cuerpo blando de larvas de insectos, siendo ésta más duradera que la marca generada por el propio peso del resto sobre el sedimento. Sin embargo, por el propio diseño del experimento, no se dejó crecer al tapete sobre los cadáveres. En nuestros ensayos, donde el tapete puede evolucionar cubriendo por completo los cuerpos, éste es un proceso rápido que aparece ya a los pocos días tras la deposición del resto sobre el tapete. Antes incluso de la total generación del sarcófago, el tapete dibuja el contorno del cadáver, si bien la formación de impresiones de gran fidelidad se ve beneficiada por el total cubrimiento del cuerpo. La interacción entre la matriz de EPS y células de pequeño tamaño con su superficie permite que cualquier estructura superficial de los cadáveres quede impresa en la cara interna del sarcófago, como el relieve de las escamas de pez (Fig. 4.4E y F), las verrugas del tegumento de la rana (Fig. 4.5D) o los microvellosidades de la superficie de una mosca (Fig. 4.3B y C). En el caso de que se produjese la mineralización del tapete, los moldes formados podrían mantenerse y, por lo tanto, preservarían la morfología externa de tejidos blandos. Este fenómeno explicaría tanto los fósiles de Ediacara como también algunos otros de cuerpo blando encontrados en Enspel (Oligoceno, Alemania) (Toporski et al. 2002). De hecho, nuestros experimentos permitieron la observación de una fina capa mineralizada sobre la cara superior de los ejemplares (capítulo 6), por lo que los moldes que se hubiesen formado fruto del contacto del tapete con el cadáver podrían conservarse durante más tiempo. Esto podría explicar, por ejemplo, la preservación de las impresiones de las estructuras anatómicas de *Pelecanimimus* (Briggs et al. 1997).

Un mecanismo esencial para la conservación de los moldes generados por los tapetes se basa en su capacidad para cohesionar el sustrato. Las estructuras sedimentarias de origen microbiano o MISS, que se explican mediante la presencia de estas comunidades, han sido halladas en numerosos yacimientos de muy diferentes épocas (e.g. Rawnsley Quarzite, Ediacárico, Australia [Gehling 2000]; Montagne Noire, Ordovícico, Francia [Noffke 2000] o Bahar Alouane, Cuaternario, Túnez [Noffke et al. 2001]). La compactación daría lugar a la formación de arrugas y texturas en la superficie de los sedimentos (Hagadorn y Bottjer 1997), pero en presencia de un cadáver y/o de una huella o traza de animal favorecería la preservación de la marca formada sobre el tapete. La existencia de tapete microbiano bentónico explicaría la preservación excepcional de huellas y/o impresiones fósiles mediante la bio-estabilización del fondo, lo que protegería al fósil de la erosión (Noffke et al. 2008), induciendo además la cementación del sedimento (Carmona et al. 2012).

MODELO DE PRESERVACIÓN EN TAPETES MICROBIANOS

En 1985, tras un extenso análisis del registro fósil de numerosos yacimientos paleontológicos, Seilacher y colaboradores propusieron el **triángulo de preservación** como explicación para la formación de yacimientos excepcionales o Konservat Lagerstätten (capítulo 1). Sin embargo, de acuerdo a los datos experimentales de los que se dispone actualmente, se puede afirmar que la participación de los tapetes microbianos en los procesos de formación de este tipo de yacimientos ha podido ser subestimada en aquellos casos en los que estuvieron presentes. En un inicio, la acción de esta comunidad microbiana, interpretada por estos autores como **sellado bacteriano**, conformaba uno de los tres mecanismos para la preservación, junto con el **ambiente húmedo** y el **enterramiento**. Nuestros resultados han demostrado que, en presencia de tapetes, los cadáveres son enterrados de manera activa y rápida dando lugar a la formación de un sarcófago que, además, mantiene la humedad de los tejidos (como se observa mediante RMI en peces de hasta 27 meses, capítulo 2). Por lo tanto, en aquellos casos en los que estas comunidades han participado en las etapas tempranas de fosilización, el mecanismo de preservación no responde al modelo de Seilacher et al. (1985), ya que los factores de humedad y enterramiento se ven influenciados, controlados y determinados por los tapetes microbianos. En este caso, la formación de los Konservat Lagerstätten en los que estuvieran presentes estas comunidades bentónicas respondería más a un triángulo en el que los tres factores principales: Sellado bacteriano, estancamiento y enterramiento rápido, no actuarían como factores independientes, sino que todos ellos estarían pivotando alrededor del baricentro que serían los tapetes microbianos como se muestra en la figura 7.2.

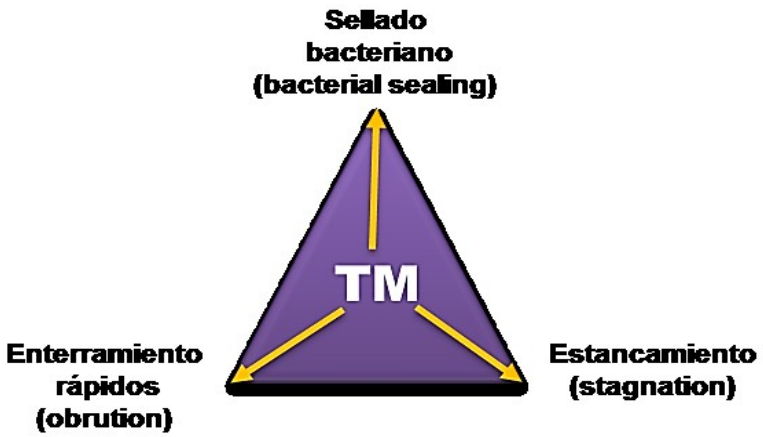


Fig. 7.2: Modificación del triángulo de preservación de Seilacher et al (1985) en el que se muestra la influencia de los tapetes microbianos en los tres factores (sellado bacteriano, enterramiento y estancamiento)

A la hora de comprender cómo puede llevarse a cabo la preservación mediante tapetes microbianos, hay que tener en cuenta las características ambientales que debería presentar el entorno en que posteriormente se generó el yacimiento. De acuerdo con las condiciones en las que actualmente se encuentran estas comunidades fototróficas, su desarrollo estaría restringido a ambientes acuáticos someros con aguas claras, ya que su crecimiento está determinado por la incidencia de la luz, como humedales, lagunas y orillas lacustres o marinas. Además, estos sistemas deberían presentar algunas características físico-químicas que restrinjan el crecimiento de sus depredadores como alto pH, altas concentraciones iónicas, etc. Mientras que un animal que se desenvuelva en el agua sufriría directamente tras su muerte los procesos derivados de su cubrimiento por los tapetes, un ejemplar que se encuentre en tierra firme estaría alejado de estas comunidades. Por lo tanto, para que pueda preservarse un animal terrestre, como es el caso de dinosaurio terópodo *Pelecanimimus* (Briggs et al. 1997), éste debe morir en la proximidad de un sistema acuático o, en su defecto, ser transportado hasta él. Este proceso ha de ser tenido en cuenta para posteriores reconstrucciones, ya que el cadáver puede sufrir diversas transformaciones biostratinómicas a lo largo de este transporte (descomposición, erosión, desarticulación, etc.). En consecuencia, la preservación mediante tapetes no afectaría por igual a organismos terrestres o acuáticos. Una vez en el agua habría que diferenciar entre los organismos según su tamaño. Los más pequeños, como insectos, tenderían a quedar en la superficie debido a la tensión superficial. Sin embargo, se ha descrito que la formación de biofilms microbianos simples a su alrededor, además de evitar la desarticulación, facilitarían su hundimiento y, por lo tanto, acortarían su fase de flotación (Gall 2001, Peñalver-Mollá 2002). En experimentos de actuotafonomía llevados a cabo con diferentes especies de peces, incluyendo ejemplares de gran tamaño (entre 47 y 60 cm), se observó que, aunque la región caudal podía presentar cierta flotabilidad, el contacto del ejemplar con el fondo era casi constante y los cadáveres quedaban totalmente depositados sobre los sedimentos a los pocos días, incluso aunque se encontrasen en agua salada (Martín-Abad 2015). Sin embargo, estos experimentos demostraron que durante la descomposición los ejemplares tendían de forma natural a desarticularse incluso en ausencia de flotación. En el caso de sistemas con tapetes microbianos, debido a su rápido crecimiento, los cuerpos se encontrarían parcialmente atrapados en el fondo a los pocos días tras su deposición, ya que los filamentos de cianobacterias crecen de forma eficaz sobre cualquier tipo de cadáver o superficie expuesta (Fig. 7.3). Además, la comunidad microbiana en su conjunto genera EPS que favorecen la inmovilización y la preservación de los cuerpos.

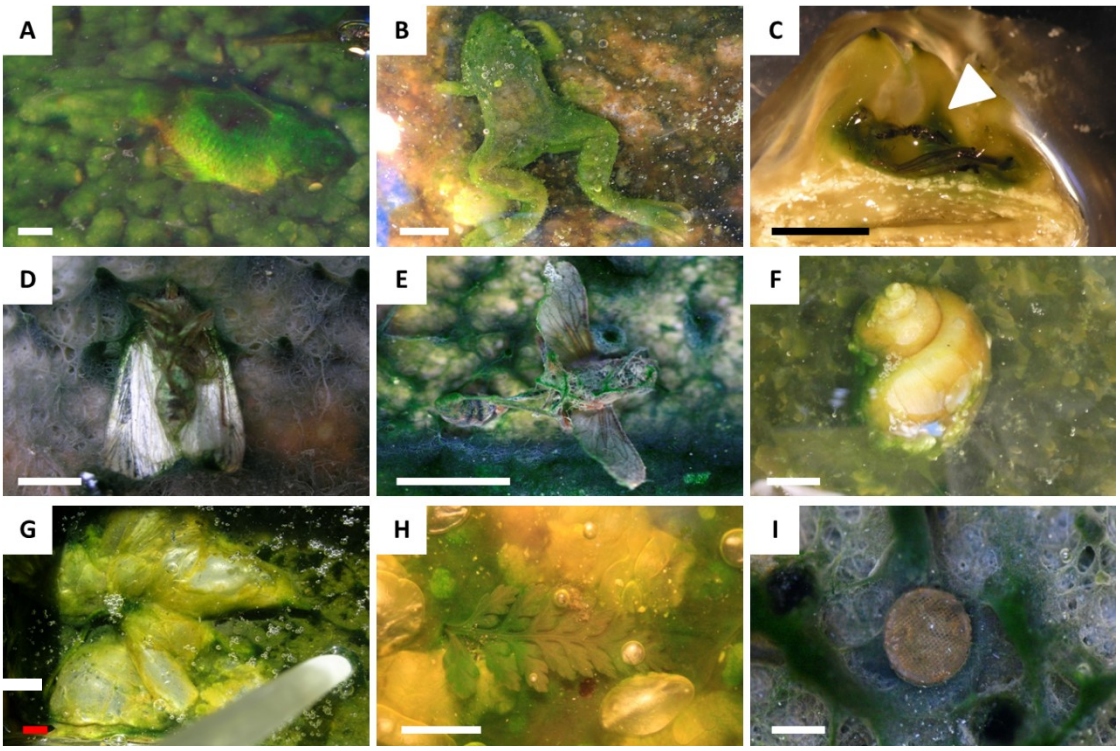


Fig. 7.3: Formación de la envoltura microbiana y fijación a la superficie del tapete de diferentes cuerpos y objetos. Entre paréntesis se detalle el tiempo transcurrido tras su deposición sobre la superficie del tapete hasta el momento en que se tomó la foto. (**A**) Ejemplar de carpa roja (*Carassius auratus*) (28 días). (**B**) Rana *Xenopus laevis* (25 dias). (**C**) Sección sagital de una mosca (*Musca domestica*) embebida en tapete microbiano (9 días). **(D)** Heterócero (10 días). (**E**) Avispa de la subfamilia Eumeninae sobre el tapete microbiano con filamentos de cianobacterias que comienzan el proceso de cubrimiento y formación del sarcófago (17 días). (**F**) Ejemplar de *Pomacea canaliculata* en el que se aprecia el crecimiento de una capa verde en su superficie (13 días). Como ejemplos del crecimiento del tapete sobre otros cuerpos diferentes a animales, se presentan fotos de la flor de una orquídea (40 días) (**G**) y la de una hoja del helecho *Davallia canariensis* atrapadas en su superficie (10 días) (**H**). (**I)** Rejilla de cobre para TEM (14 días). A pesar de tratarse de un elemento inerte compuesto por un metal pesado, el tapete también crece atrapándolo y recubriendo su superficie. Escalas: A-H: 1 cm; I: 0,5 cm.

Una vez recubierto el cadáver, la comunidad microbiana que ha formado el sarcófago continúa creciendo activamente en condiciones naturales. A medida que se deposita sedimento en su superficie, las poblaciones del tapete tienden a crecer hacia la luz, por lo que el cuerpo iría quedando progresivamente enterrado bajo una capa cada vez más gruesa. El grosor de la comunidad microbiana jugaría un papel importante en la protección de los restos frente a factores externos que pudieran alterarlos. Además, si tuviera lugar la mineralización de la cara interna del sarcófago en torno al cadáver se facilitaría su conservación tridimensional. Esto explicaría cómo pudieron producirse los fósiles que mantienen la estructura 3D como los encontrados en Las Hoyas (Briggs et al. 1997), o en Messel (Franzen et al. 2015, Vitek et al. 2013). El modelo de preservación en tapetes expuesto a lo largo de este apartado se resume en el esquema presentado en la figura 7.4

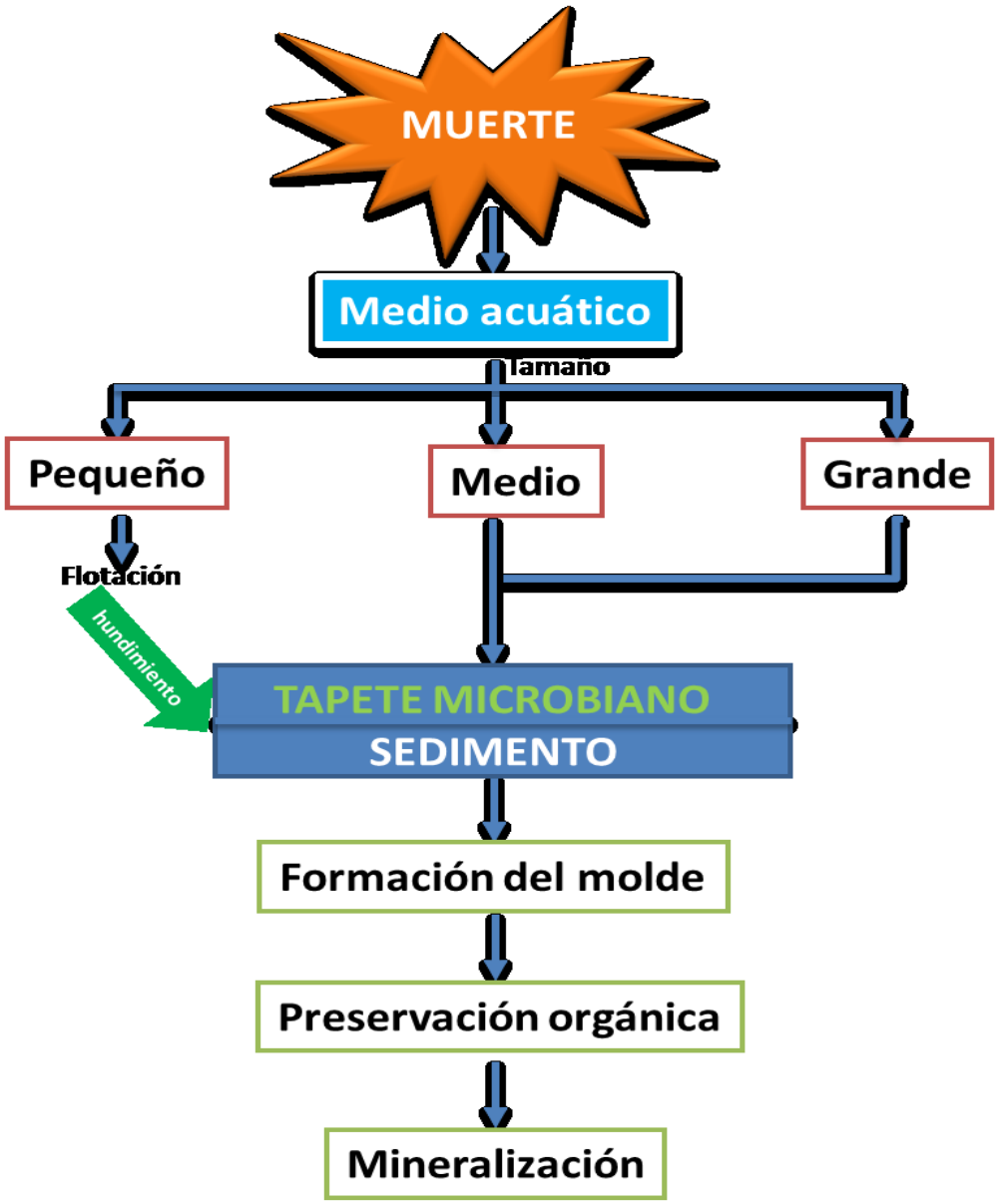


Fig. 7.4: Modelo de preservación en tapetes microbianos tras la muerte de un animal en un medio acuático cuyo fondo se encuentre tapizado por tapetes microbianos.

EVIDENCIAS ACTUOTAFONÓMICAS CON TAPETES MICROBIANOS, UNA AYUDA EN LA INTERPRETRACIÓN DE FÓSILES

En base a los resultados obtenidos, se puede tratar de explicar los mecanismos que participaron en la formación de aquellos fósiles que estuvieran relacionados con tapetes microbianos. Como se especifica en el apartado anterior, un primer aspecto importante a tener en cuenta es la paleoecología del yacimiento en el que aparecen los fósiles. Los depósitos de Las Hoyas o Libros, por ejemplo, Konservat Lagerstätten cuyos fósiles excepcionales se preservaron presumiblemente gracias a la presencia de tapetes microbianos, han sido descritos como ecosistemas acuáticos, relativamente someros y con cierta estacionalidad, un ambiente coherente con la presencia de estas comunidades bentónicas.

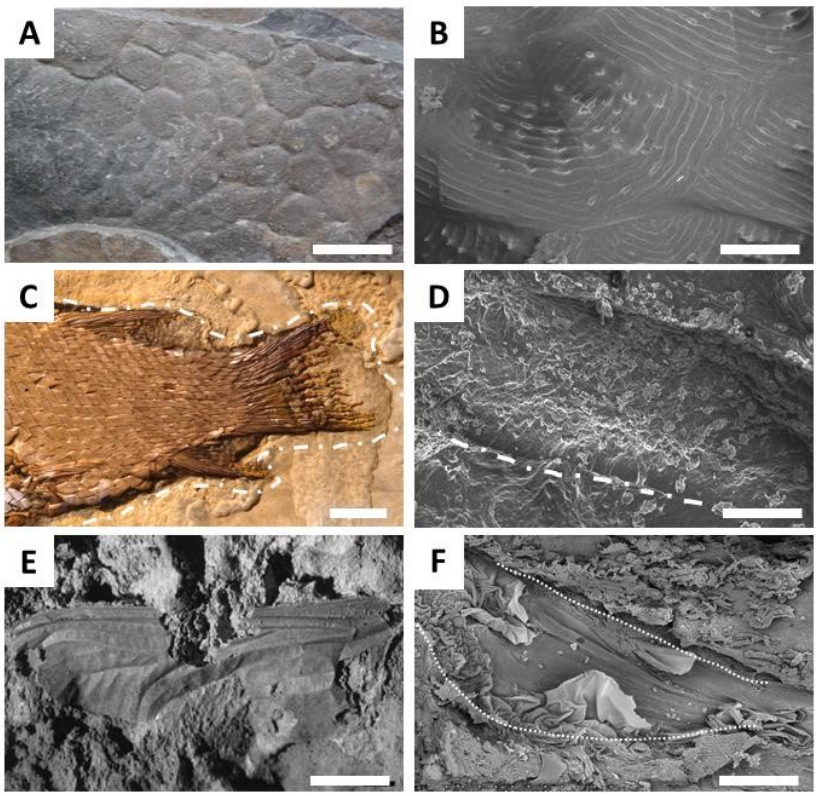


Fig. 7.5: Comparación de fósiles con impresiones obtenidas de forma experimental en tapetes microbianos. (**A**) impresión de la piel de un saurópodo (Kim et al 2010). (**B**) impresión de escamas de pez en la superficie del tapete (capítulo 4); (**C**) ejemplo de preservación del contorno de un animal (*Lepidotes microrhis*, Las Hoyas). (**D**) molde de la pata posterior de una rana formado sobre el tapete (capítulo 4). (**E**) impresión en carbonato de ala de anisóptera (ejemplar IPM-R.08356) encontrado en Auriol (Francia) (Martínez-Delclòs et al 2004). F: ejemplo de impresión del ala de una mosca sobre tapete microbiano (capitulo 4). Escalas: A: 2 cm; B: 200 µm; C: 1 cm; D: 100 µm; E: 5 mm; F: 300 µm.

Uno de los aspectos en los que estarían implicados los tapetes microbianos y que encuentra un firme apoyo con nuestros resultados es la formación de impresiones, como se ha discutido anteriormente. Dicha preservación ha sido imprescindible para

conocer diversas estructuras anatómicas como el relieve de la piel de saurópodos de los yacimientos de Haman y Jindong (Cretácico Inferior, Corea del Sur [Kim et al. 2010]) (Fig. 7.5A) o la fauna de Ediacara (Vendiense [Gehling 1999, Narbonne 2005]). Estas impresiones en los tapetes microbianos han sido observadas de forma experimental, llegando a generar moldes de las verrugas de la superficie del tegumento de ranas (Figuras 3.3 y 4.5), impresiones de escamas de pez (capítulo 4 y Fig. 7.5B) e incluso de estructuras más pequeñas como setas y sétulas de moscas (ver más adelante). De hecho, en algunas ocasiones, las huellas preservadas incluyen restos visibles de las vainas de las cianobacterias del tapete, siendo un testimonio de la participación de esta comunidad microbiana en la cementación y estabilización de la superficie en la que se forma el molde (Fernández y Pazos 2013). En algunos casos, la matriz del tapete puede mineralizar en la zona de contacto con el cadáver (capítulo 6, Fig. 6.9), como se ha observado en Las Hoyas en el caso de numerosos ejemplares de peces (la figura 7.5C muestra un fósil de *Lepidotes microrhis* de dicho yacimiento) o en el de la impresión de la bolsa gular de *Pelecanimimus*, aunque en este ejemplo el tapete se preservó mediante fosfatización (Briggs et al. 1997). Experimentalmente se ha demostrado que en el tapete microbiano pueden formarse moldes de los cuerpos (Fig. 7.5D) e incluso impresiones de su superficie de gran delicadeza y detalle que permiten, por ejemplo, la preservación de las huellas dejadas por el ala de un insecto (capítulo 4). Aunque estas marcas son puramente orgánicas, con el tiempo podrían mineralizarse, como ocurre en nuestros experimentos en la zona de contacto entre el pez y el tapete después de 5 años (capitulo 6). En tal caso, se podría mantener el grado de finura en los detalles lo que aclararía cómo se pudieron formar fósiles como los hallados en el yacimiento de Auriol (Holoceno, Francia), donde se encontraron moldes de alas de libélula (Martínez-Delclòs et al. 2004) (Fig 7.5E) de forma similar a lo observado en nuestros experimentos (Fig 7.5F).

La preservación de los cadáveres en sentido estricto, i.e. los tejidos propios del animal y no un molde de su forma, también puede explicarse en algunos yacimientos por la influencia de los tapetes microbianos. Existen diversos ejemplos de preservación de tejidos blandos en el registro fósil, como en el caso de restos de músculo fosfatizado encontrados en ejemplares de cangrejo cacerola *Mesolimulus* en el yacimiento de Nusplingen (Jurásico, Alemania [Briggs et al. 2005]) (Fig 7.6A) o conservados en una matriz carbonatada como en los peces hallados en Múzquiz (Cretácico, México) (Riquelme et al. 2013). En estos casos, la organización en fibras y el bandeado de las mismas es similar a lo encontrado en nuestros experimentos en algunas de las capas de tejido preservado en ranas de 540 días (Fig. 7.6B). También se encuentra conservado, aunque en este caso como carbonatos de Fe, el músculo de *Pelecanimimus* de Las Hoyas, junto con algunas de las células que conformaban el sarcófago que recubriría su piel y sus músculos (Briggs et al. 1997) y que quedaron sobre la superficie del ejemplar. En nuestros experimentos, la única estructura blanda que se ha conservado en forma de carbonato, en este caso de Ca, ha sido una parte del cerebro de ranas adultas (Fig. 7.6D y F). Sin embargo este resultado es especialmente interesante porque esa misma clase de precipitados aparece en los cerebros de renacuajos (McNamara et al. 2010) (Fig. 7.6C y E) o de ejemplares adultos de anuro (McNamara et al. 2009) del yacimiento de Libros

(Mioceno, Teruel). Además, en ocasiones puede llegar a preservarse un tejido aún más lábil que las fibras musculares como es la médula ósea. La conservación del interior de los huesos observada en nuestros experimentos, en los que se describen restos de médula en ranas después de un año (Fig. 4.7) o tejido hematopoyético dentro de huesos de 1.5 años (Fig. 7.6H) explicarían la formación de fósiles con médula ósea en su interior como los que se han encontrado en Libros (McNamara et al. 2006) o la aparición de células sanguíneas en huesos fósiles de dinosaurio de 75 Ma (Bertazzo et al. 2015) (Fig. 7.6G).

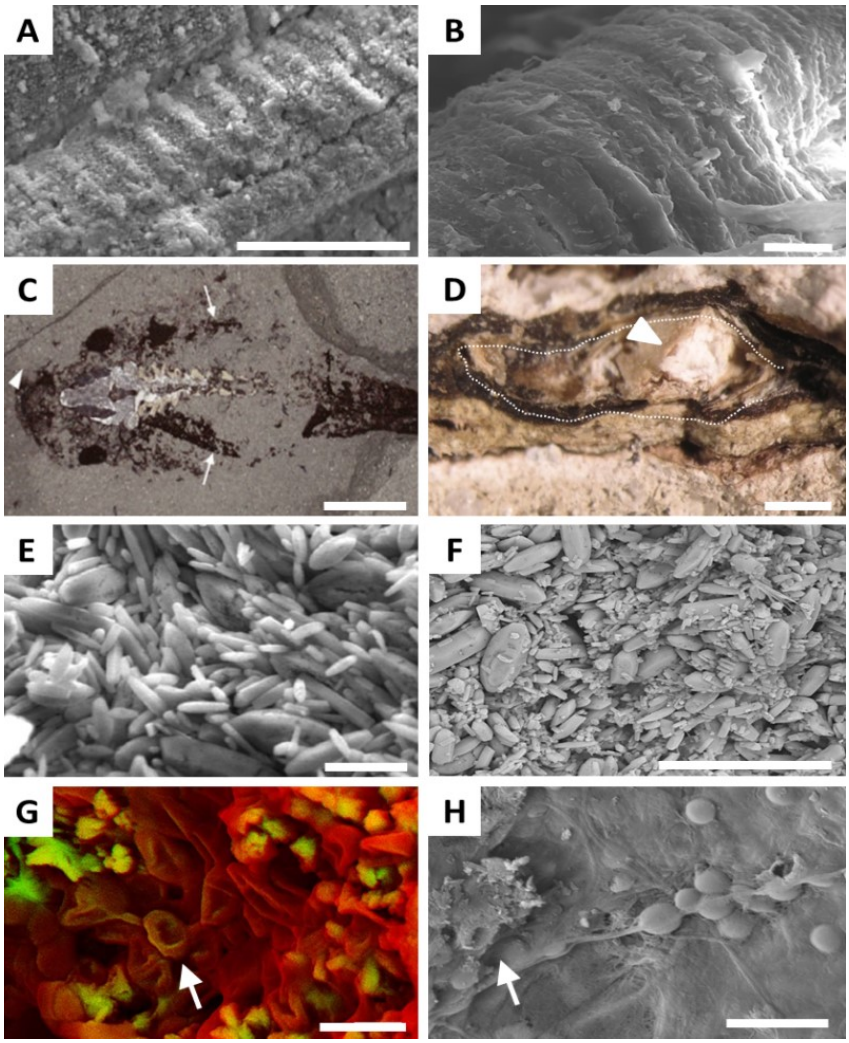


Fig. 7.6: Ejemplos de fósiles de tejidos blandos y su posible análogo obtenido de forma experimental en tapetes microbianos. (**A**) músculo de cangrejo cacerola fosfatizado (Briggs et al. 2005). (**B**) Detalle de fibras musculares de una rana después de 1.5 años en el tapete (capítulo 3). (**C**) Renacuajo fósil del yacimiento de Libros en donde el cerebro se observa como una mancha blanca (Mcnamara et al. 2010). (**D**) Sección sagital de la cabeza de una rana envuelta por el tapete microbiano (3 años). Obsérvese la mancha blanca en el lugar ocupado por el lóbulo óptico inicialmente. (**E**) Detalle de los cristales de carbonato de Ca en el cerebro del renacuajo de C (McNamara et al 2010). (**F**) cristales de carbonato de Ca del cerebro de una rana tras 3 años en el sarcófago (capítulo 3). (**G**) Células sanguíneas (flecha) en el interior de un hueso de dinosaurio (Bertazzo et al 2015). (**H**) Tejido hematopoyético con células sanguíneas (flecha) dentro de un hueso de rana después de 1.5 años en el sarcófago. Escalas: A: 10 µm; B: 10 µm; C: 20 mm; D: 0.5 cm; E: 10 µm; F: 10 µm; G: 5 µm; H: 5 µm; I: 2 µm; J: 5 µm. A, B, E-H son imágenes obtenidas con SEM.

Por otro lado, el sarcófago formado por los tapetes se ha mostrado como un aspecto clave en la inhibición de la desarticulación y el mantenimiento de la forma corporal en los animales experimentales, independientemente del tamaño (dentro de un rango entre 0.5 y 3 cm aproximadamente) y del grado de dureza (desde más duro –moscas- a más blandos –ranas-). Esta capacidad de conservación permitiría explicar la excepcional integridad y grado de articulación de los fósiles de algunos yacimientos. Por ejemplo, en numerosos casos, los ejemplares de peces encontrados en Las Hoyas presentan la totalidad de las escamas perfectamente organizadas (e.g. los ejemplares de *Sinama zdanski* (IVPP 1106) o de *Calamopleurus cylindricus* (FMNH F14348b), ver Fig. 1.9 y 1.10 en Martín-Abad 2015), de forma similar a lo que se observa mediante SEM en los peces de nuestros experimentos (e.g. Fig. 2.2 y 4.4). En otros ejemplares fósiles encontrados en Las Hoyas se aprecia, la perfecta articulación de los radios de las aletas (Martin-Abad, 2015) análogamente a lo hallado en algunos de nuestros ejemplares (Fig. 2.2). Además de favorecer la articulación y conexión de los restos, la cubierta microbiana puede evitar la degradación de los huesos y mantener estructuras duras más delicadas. En nuestros experimentos, tanto las espinas de los peces como los huesos de las ranas permanecen casi inalterados dentro del sarcófago (capítulos 2 y 3 y Fig. 7.7B). Su aspecto es similar al de los huesos de saurios de diferentes edades (incluyendo ejemplares de *Tyrannosaurus rex* de hasta 68 Ma o de *Brachylophosaurus canadensis* de 78 Ma descritos por Schweitzer et al [2007] (Fig. 7.7A), o de ejemplares de ornistiquios y saurisquios de 75 Ma reseñado por Bertazzo et al [2015]). En el caso de las moscas, éstas mantenían estructuras delicadas como omatidios o setas (Fig. 7.7D, F y H y Fig. 7.8B, D y F respectivamente). Este grado de protección explicaría la formación de fósiles como los que se encuentran en el yacimiento de Crato (Cretácico, Brasil [Barling et al 2015]) que mantienen la articulación y aún se pueden apreciar las setas en su superficie (Fig. 7.7C, G e I), o el yacimiento de Kishenehn (Eoceno, USA), donde aparecen insectos embebidos en la matriz de tapete microbiano (Greenwalt et al. 2015) (Fig. 7.7E). También se han encontrado fósiles que presentaban, al igual que las moscas de nuestros experimentos, omatidios preservados y perfectamente organizados como en el caso de trilobites encontrados en diferentes yacimientos que van desde el Cámbrico (-542Ma) hasta el Carbonífero (-299Ma) (Clarkson et al. 2006) (Fig. 7.8A) o de artrópodos del yacimiento de Crato (Brasil) (Barling et al. 2015) (Fig. 7.8C y E) o de Bahía Emu (Cámbrico, Australia [Lee et al. 2011)].

En ciertas ocasiones, además del cadáver preservado, encontramos microorganismos del sarcófago adheridos a la superficie del ejemplar, como en *Pelecanimimus* de Las Hoyas (Briggs et al. 1997) o en fósiles de Nusplingen (Jurásico, Alemania) (Briggs et al. 2005). Para que estas células hayan podido llegar hasta la actualidad debieron sufrir un proceso de mineralización. Así, en fósiles de Messel (Eoceno, Alemania) (Franzen et al. 2015) o de La Voulte-sur-Rhône (Jurásico, Francia) (Robin et al. 2015) se han podido analizar células que han colonizado la parte externa de los cadáveres en forma de biofilm. En ambos casos parece que la actividad de los microorganismos podría explicar la calidad de la preservación. El fósil descrito por Franzen et al (2015) es un feto de *Eurohippus messelensis* que fosilizó en el interior de la madre y parte de su cuerpo está recubierto por células con forma bacilar. Aunque no se puede descartar que existiese

una colonización desde los tapetes microbianos, ya que su presencia en el yacimiento ha sido confirmada (Franzen 1985), también pudiera tratarse de poblaciones provenientes de la microbiota intestinal. Sin embargo, en el caso del yacimiento de Francia, los microorganismos se encuentran en la superficie externa y habrían calcificado adheridos a cadáveres de gamba, quedando epibionte y basibionte preservados como un conjunto (Robin et al. 2015). Este tipo de colonización de los cadáveres por células de pequeño tamaño es análoga a la que se ha descrito en nuestros experimentos (capítulo 4). Estas células generan los moldes o replican estructuras superficiales del cuerpo, mientras que en la parte más externa del sarcófago se encuentran filamentos más gruesos que dan cohesión a la cubierta. Por otro lado, la formación de minerales en la cara externa de los cadáveres a consecuencia de la actividad microbiana, como la calcificación de las bacterias sobre las gambas de La Voulte-sur-Rhône, también ha sido observada en huesos de vertebrados de Cerro de la Garita en Teruel (Mioceno, España). En este caso, los huesos fósiles presentan una cubierta de calcita que parece surgir a partir de la degradación localizada de las capas externas del hueso (Pesquero y Fernandez-Jalvo 2014). Aunque en nuestros experimentos no hemos observado la aparición de esta capa de calcita, sí que se ha comprobado un proceso de transformación similar en la superficie de las escamas superiores y en las espinas de las aletas de los peces que dio lugar a la formación de un silicato de magnesio (capítulo 6). Este proceso podría explicar la aparición de carbonato cálcico rodeando y delineando los restos fósiles de renacuajos en el yacimiento de Libros (Mcnamara et al. 2010). Como se ha descrito anteriormente, el mineral que se forma depende de múltiples factores difíciles de simular y/o dilucidar, pero las condiciones químicas para la formación tanto de silicatos de magnesio en nuestros experimentos, como de carbonatos en el caso de las bacterias de La Voulte-sur-Rhône o Libros, pueden ser explicadas por la actividad de los tapetes microbianos.

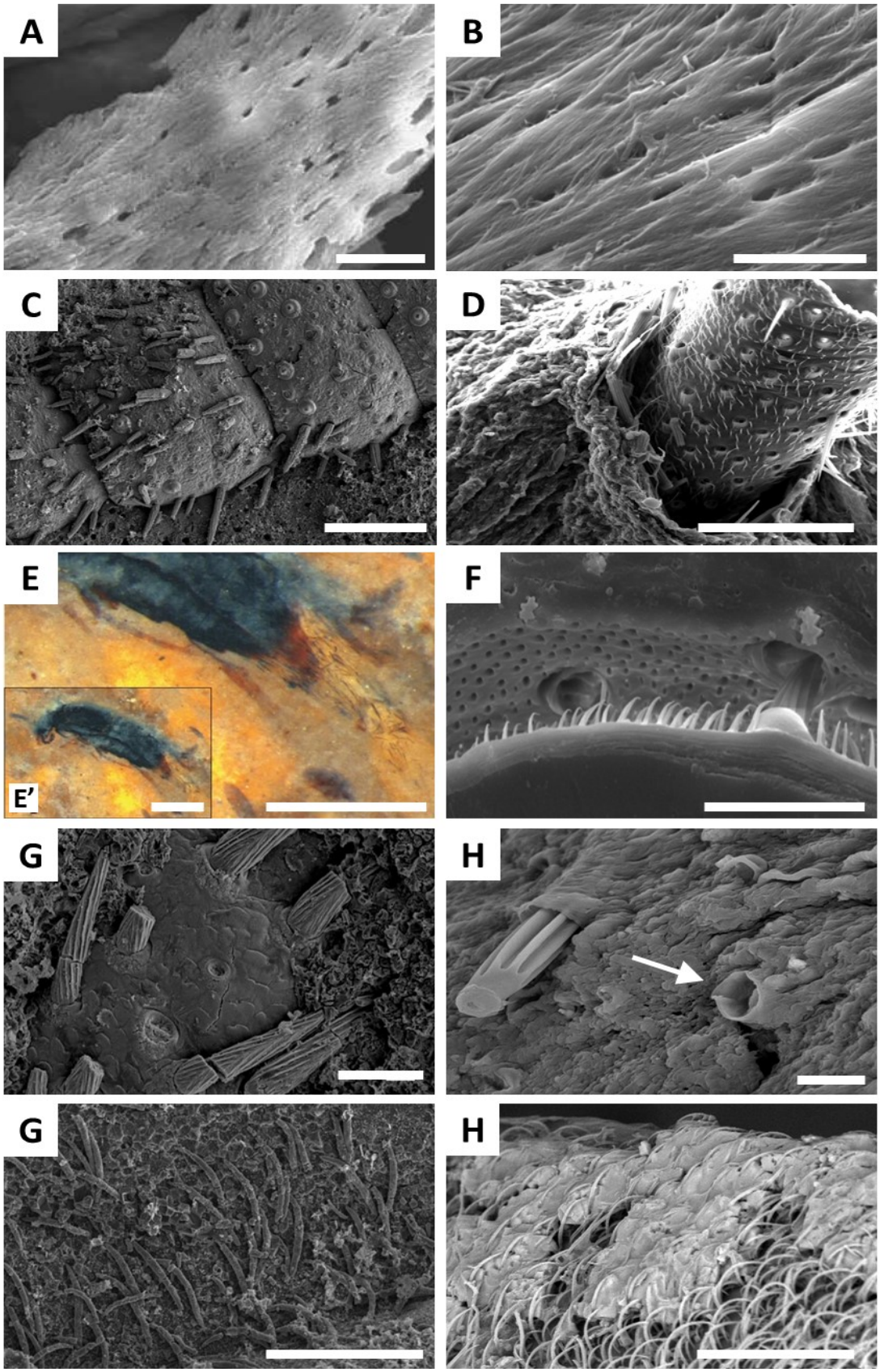

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Fig. 7.7 (página anterior): Comparativa de ejemplos de fósiles articulados y observaciones realizadas en experimentos con tapetes microbianos. (**A**) Superficie de un hueso de dinosaurio del jurásico (Schweitzer et al 2007). (**B**) Superficie de hueso de rana tras 3 años en tapete. (**C**) fósil de blatodeo (JW291) del yacimiento de Crato (Barling et al 2015). (**D**) Pata de mosca recubierta por tapete microbiano. (**E**) detalle de fósil de coleóptero envuelto en tapete microbiano mineralizado (Greenwalt et al 2015) en el que se pueden apreciar las setas preservadas (**E'** muestra la localización de la imagen mostrada en **E**). (**F**) Sección del sarcófago con una mosca en su interior (8 meses) en el que se aprecian las setas y las marcas en el tapete dejadas por las sétulas. (**G**) Fósil de blatodeo (NBRL059) en el que se aprecian las setas aún articuladas (Barling et al 2015). (**H**) Setas de mosca en el tapete microbiano. Cuando la seta es retirada de la envuelta, el sarcófago mantiene la vaina que lo recubría (flecha). (**I**) Fósil de caelífero (NBRL051) con setas preservadas (Barling et al 2015). (**J**) Setas de mosca sobre la que se puede observar la matriz de EPS producida por el tapete microbiano (12 meses). Escalas: A: 5 µm; B: 5 µm; C: 20 mm; D: 10 µm; E: 1 mm; F: 50 µm; G: 20 µm; H: 10 µm; I: 100 µm; J:

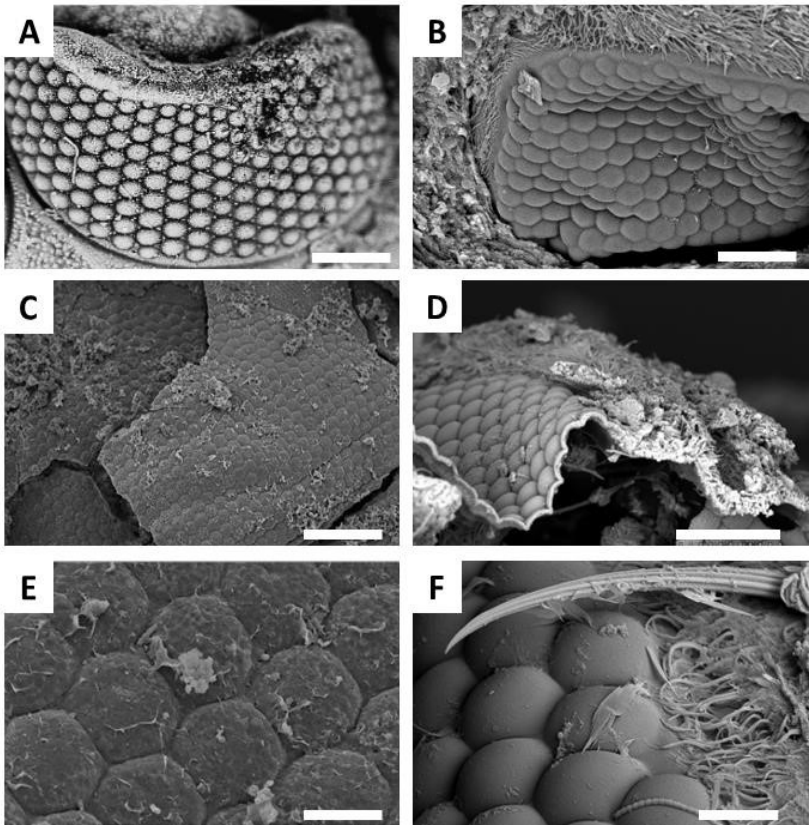

Fig. 7.8: Imágenes al SEM de fósiles de ojo compuesto y su comparación con ejemplos experimentales de preservación de ojo compuesto en tapete microbiano (**A**) Ojo de trilobite *Calyptaulax brongniartii* -Portlock, 1843 (Ayrshire, Scotland)- (Clarkson et al 2006). (**B**) Mosca en el tapete microbiano después de 5.5 años. (**C**) Ojo fracturado de caelífero (Barling et al 2015). (**D**) Lámina de omatidios fracturada en ejemplar de mosca tras 12 meses en tapete. (**E**) detalle de **C** (Barling et al 2015). (**F**) Detalle del grado de preservación de los omatidios en mosca cubierta por el tapete (5.5 años). Escalas: A: 1 mm; B: 50 µm; C: 150 µm; D: 100 cm; E: 20 µm; F: 20 µm.

PERSPECTIVAS FUTURAS

Resulta evidente, tras nuestros trabajos con tapetes microbianos, que estas comunidades pudieron mediar en numerosos aspectos tafonómicos hasta la formación de determinados fósiles. Sin embargo, es también indiscutible que esto no es más que un pequeño paso y que muchas de las transformaciones que conducen a la preservación están aún por desvelar. A continuación se harán una serie de consideraciones y propuestas que pudieran hacernos avanzar en el conocimiento de los procesos que darían lugar a la formación de un fósil.

La formación de un sarcófago por parte del tapete microbiano ha demostrado ser un elemento esencial para preservar los cadáveres y evitar su desarticulación durante un tiempo considerablemente más largo que en los controles. Sin embargo, en el laboratorio los tapetes no crecen ni al mismo ritmo ni lo hacen de la misma forma que en su ambiente natural, al no contar con la sedimentación propia del sistema del cual proceden. Así que los procesos diagenéticos difícilmente van a poder ser simulados de forma análoga a la realidad con el tipo de experimentación que se ha llevado a cabo hasta el momento. Sin embargo, se podría intentar reproducir las posibles transformaciones químicas que pueden experimentar los restos a lo largo del tiempo geológico. En un artículo publicado recientemente, Bernard et al. (2015) estudiaron el efecto de la temperatura elevada (hasta 1000ºC) sobre un componente de la pared de las esporas de *Lycopodium clavatum* (la esporopolenina) y comprobaron sus posibles modificaciones mediante una serie de técnicas analíticas entre las que se encontraba la espectroscopía Raman. Aunque, en base a los resultados, los autores determinan que el experimento no reprodujo las modificaciones observadas en muestras de esporas fósiles y, por lo tanto, el incremento de temperatura no mimetizó de forma fiel el proceso de transformación al que se pueden ver sometidos los cadáveres durante la diagénesis, variaciones de este tipo de aproximaciones pueden ser muy útiles para intentar simular los procesos que se pueden dar durante esta etapa. Los experimentos de maduración artificial de moléculas orgánicas muestran así que el incremento en presión y temperatura de la diagénesis modifica la composición en macromoléculas de los seres vivos (Stankiewicz et al. 2000). En este contexto, es importante también hacer un seguimiento a nivel molecular de la preservación de los tejidos. Existen moléculas que son bastante resistentes a la descomposición, como la lignina o la celulosa de las paredes vegetales (van Bergen et al. 1995), mientras que otras, como los lípidos, son altamente degradables (Briggs 1999). Además, hay que tener en cuenta que las transformaciones sufridas pueden generar artefactos. Por ejemplo, la degradación de los lípidos de la pared celular puede producir compuestos alifáticos mediante polimerización *in-situ* (Briggs et al. 1998) similares a los que se esperaría encontrar en una cutícula (Gupta 2014). De hecho, experimentos que analizaron la cutícula de artrópodos en descomposición mediante resonancia magnética nuclear (NMR del inglés) observaron que la quitina había sido sustituida por otros compuestos orgánicos (Baas et al. 1995) que no provenían originalmente de ella. Por lo tanto, estudiar en profundidad cómo se preservan las moléculas orgánicas en el interior del sarcófago tiene gran

importancia para interpretar posteriormente los fósiles. Los compuestos orgánicos, especialmente los lípidos, son sensibles a la diagénesis y pueden producir señales características o biomarcadores (Engel y Macko 1993) que pueden ser detectados, por ejemplo, mediante cromatografía iónica (Briggs 1999), NMR o pirólisis con cromatografía de gases y espectrometría de masas (Py-GC/MS de sus siglas en inglés) (Gupta 2014). Si la secuencia de descomposición de los restos en tapetes microbianos se ve modificada con respecto a la que tiene lugar en ausencia de tapetes, existe la posibilidad de que los cambios que sufren los compuestos orgánicos sean también diferentes y, por lo tanto, que puedan existir biomarcadores específicos según la historia tafonómica.

Un aspecto enorme importancia en el proceso de preservación es conocer de qué manera los fósiles han llegado a convertirse en materia mineral. Resultan especialmente interesantes, por ser relativamente frecuentes entre los tejidos blandos conservados, aquellos que se encuentran fosfatizados (e.g. Martill 1988, 1990 o Briggs et al 2005) y también, aunque menos corrientes, los piritizados (e.g. Bartels et al. 1998 o Farrell et al. 2009). Sin embargo, estos tipos de precipitados no han sido los que han aparecido en nuestros experimentos. Como se describe en el capítulo 6, el sistema en el que se preservan los ejemplares, que deriva de la composición del sedimento natural de la laguna de Chiprana, favorece la precipitación de silicatos ricos en magnesio. Además, debido a la elevada concentración en Ca del agua (ver Tabla 6.1) así como al pH básico generado en el sarcófago, se puede observar también la aparición de carbonatos de Ca tanto en la superficie de los cuerpos (Fig. 2.5) como en el interior de los cráneos, sustituyendo tejidos orgánicos (Fig. 3.8). No obstante y como se ha comentado anteriormente, el tipo de mineralización autigénica depende en gran medida de las características ambientales. Así, y aunque la fosfatización de tejidos ha sido comúnmente asociada a ambientes ácidos (e.g. Briggs y Wilby 1996 o Briggs et al. 1993), algunos estudios de mineralogía y química demuestran que este fenómeno es igualmente posible en un ambiente básico como el que se ha descrito en el interior del sarcófago, siempre y cuando la solución se encuentre saturada en fosfatos (Song et al. 2002). La variación controlada en los experimentos de algunas características claves para intentar facilitar la precipitación de compuestos concretos, por ejemplo, mediante la adición artificial y regular de elementos como P, Ca o Fe al sistema de experimentación, que percolarán posteriormente al interior del sarcófago, podría favorecer la formación de fases minerales diferentes a las observadas en la presente tesis.

Como se ha demostrado a lo largo de este trabajo, la actividad microbiana juega un papel clave en muchos y muy diferentes procesos que afectan a los cadáveres y determinan la fosilización de los mismos. De hecho, los microorganismos son responsables de la descomposición de los cuerpos, pero también participan en su preservación. Por otro lado, la actividad microbiana puede contribuir a la disolución de minerales, pero también puede generar las condiciones para la precipitación de multitud de fases minerales. Toda esta variedad de procesos depende tanto de la composición poblacional como de las actividades que se lleven a cabo en la comunidad microbiana. Por lo tanto, un aspecto que puede resultar de especial importancia para comprender

los mecanismos de preservación en tapetes microbianos es la microbiología del proceso. Raff et al (2013) intentaron abordar este punto determinando las poblaciones implicadas en la descomposición y/o preservación de embriones en velos heterótrofos, mediante la inoculación de cepas conocidas de bacterias sobre los embriones analizados. La observación de cómo se preservaban o deterioraban los embriones determinaría por lo tanto la influencia que ese organismo tendría en la descomposición. El principal problema de este análisis se basa en el sesgo que supone reducir la comunidad microbiana a la suma de sus poblaciones. Como se ha explicado anteriormente (apartado 1.1), el crecimiento agregado de los microorganismos conduce a modificaciones en su fenotipo y su expresión génica (Costerton 2012, Stolz 2000). Así, analizar el efecto individualizado de cada una de las poblaciones puede estar enmascarando el de las interacciones propias del biofilm. Otra forma de conocer el tipo de poblaciones implicadas puede basarse en la secuenciación del gen 16S rRNA, como realizaron Darroch et al (2012) en experimentos con tapetes microbianos en los que se reprodujo la formación de huellas de larvas en su superficie. En este caso, el análisis genético se llevó a cabo al inicio del experimento y, por lo tanto, sirvió para determinar la composición del tapete microbiano pero no qué poblaciones pueden estar implicadas en la formación de la huella y/o la preservación de la larva. Como se ha visto en nuestros experimentos, la llegada a la superficie del tapete de un elemento alóctono, por ejemplo un cadáver, provoca una reestructuración y estimulación de la comunidad que desembocan en la formación del sarcófago. Por lo tanto, para intentar determinar qué poblaciones concretas pueden estar implicadas en el proceso tafonómico, el estudio debe tener en cuenta el aspecto temporal, realizando análisis a diferentes tiempos. Las técnicas moleculares actuales como la metagenómica, a través de la secuenciación masiva mediante pirosecuenciación (para un ejemplo de aplicación en tapetes microbianos ver Ugalde et al. 2013) o, más recientemente, la secuenciación de nueva generación realizada por Illumina (Saghaï et al. 2015), permiten obtener grandes cantidades de secuencias génicas en muy poco tiempo, aunque esto complica considerablemente su análisis posterior. Sin embargo, ni conocer el tipo de poblaciones ni todos sus genes permite saber de forma concreta qué metabolismos estarían implicados en la preservación de los cadáveres. Para conocer las funciones microbianas que se encuentran activas en cada fase del proceso sería necesario un análisis del transcriptoma de la comunidad. Esta información que desvelaría los genes que se estuvieran expresando a lo largo de las primeras etapas tafonómicas, nos permitiría correlacionar las actividades microbianas con el ambiente físico-químico así como con las fases minerales que podrían verse favorecidas a lo largo del proceso. En definitiva, quedan aún muchas incógnitas por desvelar que precisarán de todo un abanico de técnicas y enfoques para tratar de llegar, de forma cada vez más precisa, al conocimiento de cómo puede ocurrir la fosilización mediada por tapetes microbianos.

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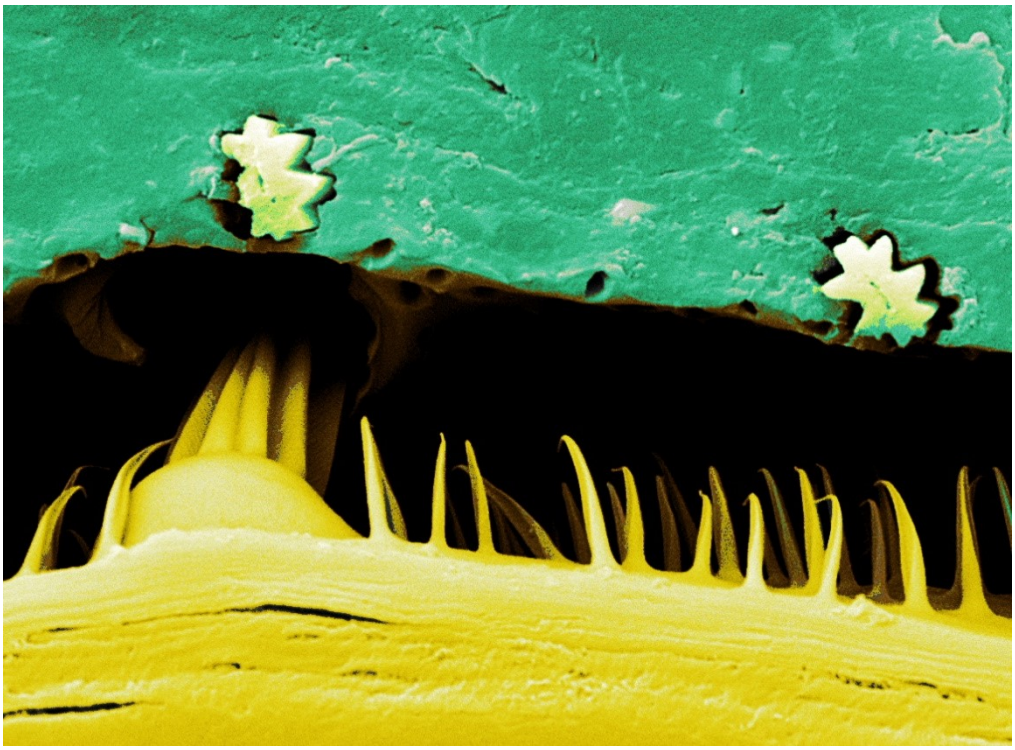
CONCLUSIONES



CONCLUSIONES

1. La comparación, mediante el estudio de variables morfométricas y cualitativas, del grado de descomposición de ranas y peces colocados sobre tapetes microbianos, ha permitido comprobar de forma sistemática su menor grado de deterioro con respecto a los controles sobre sedimento sin tapete. La presencia de estas comunidades microbianas ralentiza la descomposición de los cadáveres, preservando los restos durante largo tiempo.

2. Una vez que los cuerpos son depositados sobre la superficie de un tapete microbiano, éste comienza una etapa de activación y reestructuración de sus capas superiores que desemboca en la formación de un sarcófago que envuelve los cadáveres muy rápidamente (entre 2-4 semanas dependiendo del tamaño del cadáver y el estado del tapete). Esta cubierta confiere protección frente a la desecación, el arrastre y la desarticulación de los restos.

3. La presencia de un cadáver y su posterior recubrimiento por parte del tapete modifica las condiciones microambientales de su entorno. Se observan dos etapas en el interior del cuerpo: En la primera, que dura apenas una semana desde la deposición del resto y en donde éste aún no ha sido cubierto por el tapete, el pH y el OD varían de forma desacoplada mostrando valores relativamente ácidos de pH y muy bajos de OD. En la segunda etapa, que permanece constante a lo largo del tiempo, se genera un microambiente básico y óxico que favorece la preservación de los tejidos a largo plazo. Estas condiciones están promovidas principalmente por la actividad de las capas superiores del tapete, donde predominan los microorganismos fotosintéticos oxigénicos, por lo que su efecto en la menor degradación de los cadáveres es muy relevante.

4. En la zona de contacto del tapete microbiano con la superficie de los cuerpos se genera una impresión detallada del relieve exterior de éstos. Dicha impresión o puede copiar en negativo incluso estructuras muy pequeñas (hasta de 3-4 μm como en el caso de las sétulas de las moscas) y está formada principalmente por células de pequeño tamaño y EPS. En las zonas más

externas del sarcófago se sitúan microorganismos de mayor tamaño, confiriéndole una gran compactación y solidez. La formación de impresiones en los tapetes microbianos de tejidos lábiles como la piel, hacen posible la obtención de fósiles que permiten la descripción de, por ejemplo, la textura de la piel de dinosaurios, o la presencia de estructuras carnosas como la bolsa gular.

5. En ocasiones, la colonización por parte de los microorganismos de la superficie de los cadáveres desemboca en la replicación de órganos delicados, como por ejemplo los ojos. Estas réplicas, que son detalladas y mantienen la estructura original del órgano, también están compuestas por células de pequeño tamaño embebidas en una matriz de EPS.

6. Se ha comprobado la existencia de tejidos blandos preservados a largo plazo en cadáveres depositados sobre tapetes microbianos. En el caso de los peces se ha observado paquetes musculares a los dos años y la vejiga natatoria después de cinco años. En ranas con tres años de permanencia en los tapetes, se han conservado músculos, tendones, piel e incluso médula ósea en donde se ha identificado tanto tejido graso como hematopoyético. En moscas de 5.5 años se ha podido comprobar la existencia de algunas partes de los ovarios y del tracto digestivo.

7. Algunos tejidos blandos se han mineralizado a lo largo de los experimentos. En peces, tras 4 años en los tapetes, se formó una fase mineral de silicato rico en magnesio presente tanto en el interior de los cuerpos como sobre su cara superior. En ranas, el cerebro en su región media (el tectum) fue transformado en cristales de carbonato cálcico después de 1.5 años. Estos precipitados fueron detectados en la misma zona del cráneo en ranas de tres años.

8. Los resultados obtenidos en la presente tesis, como la preservación de tejidos blandos, la aparición de bioprecipitados bien en zonas concretas de los cadáveres bienreemplazando determinados tejidos blandos, o la formación de impresiones y réplicas por parte del tapete microbiana, pueden ayudar a entender cómo se formaron los fósiles que pertenecen a yacimientos donde pudieron desarrollarse tapetes microbianos. Por ejemplo, podrían explicar la

aparición de carbonato cálcico en el cerebro de ranas fosilizadas de varios yacimientos paleontológicos.

9. En los experimentos tafonómicos que intentaban demostrar la mineralización de tejido asociada a la descomposición, en donde únicamente participaban comunidades microbianas heterótrofas, se generaba un microambiente ácido y anóxico. Por esta razón se había asumido que estas condiciones eran necesarias para la mineralización autigénica de los tejidos orgánicos. Sin embargo, en esta tesis se ha demostrado que si la descomposición ocurre asociada a la actividad de los microorganismos que forman parte de un tapete microbiano fotoautotrofo (dominado por cianobacterias) el microambiente que se crea es óxico y alcalino, pese a lo cual, los tejidos son conservados e incluso algunos mineralizados - En consecuencia, el modelo de preservación debe replantearse al menos para aquellos casos en que estuvieron presentes estos ecosistemas microbianos.

10. Se ha demostrado que los tapetes microbianos fotótrofos influyen sobre las primeras etapas de fosilización en al menos, las dos vías de preservación de tejidos del cadáver admitidas hasta el momento: la conservación orgánica y la mineralización de tejidos mediada por microorganismos, por lo que el papel en la conservación de los restos, cuando estas comunidades estuvieran presentes habría sido clave.

11. Los notables resultados obtenidos a lo largo del desarrollo de esta tesis han abierto numerosas vías de investigación que pueden esclarecer cómo se formaron algunos tipos de fósiles. Se requieren más experimentos para precisar, por ejemplo, cómo se transforman las impresiones o las réplicas orgánicas formadas por los microorganismos en estructuras fosilizadas o para explicar cómo se producen determinados tipos de bioprecipitados sobre estructuras o tejidos concretos, así como para entender cuáles son las condiciones microambientales que determinan, en cada caso, la aparición de uno u otro tipo de precipitado. Por último, conocer las variaciones en las poblaciones microbianas durante la formación del sarcófago permitirá comprender mejor estas variaciones en las condiciones ambientales del sistema.

CONCLUSIONS

1. The presence of microbial mats delays the decay of animal carcasses, allowing a better preservation. The study of decay of frogs and fish by morphometric and qualitative parameters confirmed the lesser damage suffered by corpses in mats in comparison with controls over sediment.

2. The placement of bodies over the mat is followed by the stimulation and reorganization of the upper layers, leading to the formation of a sarcophagus that quickly covers carcasses (2-4 weeks depending on the body size and mat conditions). This coverage protects against dehydration, drag and disarticulation.

3. The presence of a body and the subsequent coverage by the mat modifies the microenvironmental conditions of the surroundings. Two different stages have been described. The first one is one-week standing begins after the placement of carcasses over mat and is characterized by anoxia and slightly acid pH. During the second stage, which is long standing, the environment is oxic and pH is basic. These conditions should favour tissue preservation in the long time and are mostly determined by the activity of the upper layers of the mat, made mainly by oxygenic photosynthetic microorganims.

4. The intimate contact of the sarcophagus with the surface of the body generates a detailed impression of the external shape of carcasses. This imprints, mainly made by small cells and EPS, are able to copy in negative even small structures such fly setulae (3-4 μm). The external zones of the sarcophagus are built by bigger cyanobacterial filaments responsible for the huge coherence of the coverage. Impression of labile structures are at the base of the generation of several fossils which are essencial in order to describe, for instance, the skin of dinosaurs or the existence of soft external structures as the throat pouch.

5. In occasions, microbial colonization of the carcass lead to the replication of soft external organs such as the eyes. These detailed replicates maintain the original structure and are also made by small cells embedded in an EPS matrix.

6. We have shown the preservation of inner soft tissues in mats in the long term. In the case of fish, muscle bundles are present after two years and the swim bladder is still intact even after five years. In frogs which spent three years in the sarcophagus we detected the existence of muscles, tendons, skin and bone marrow still composed by both the fatty and the hematopoyetic tissue. Flies showed to preserve inner organs such as ovaries and digestive tract after 5.5 years inside the mat.

7. Several soft tissues have mineralized in the course of the experiment. In fish, a Mg-rich silicat phase was formed inside and outside the body after four years. In frogs, the midbrain (the tectum) was replaced by calcium carbonate crystals after 1.5 years. Similar mineralized tissue was detected in the same area of the skull in frogs after three years.

8. The outcomes of the present work, i.e. the preservation of soft tissues, the occurrence of bioprecipitates in targeted zones and the generation of impressions and replicates, are useful in order to understand the fossil record of those sites where microbial mats would have developed. For instance, this could explain carbonate mineralization of brains observed in fossilized frogs from several Spanish deposits.

9. Previous taphonomic experiments trying to explain mineralization associated with decay and performed with heterotrophic microbial communities, stated that the environment for the occurrence of bioprecipitates linked to preservation had to be anoxic and acid. However, the results exposed in the present thesis have shown that decomposition in presence of cyanobacterial microbial mats results in an oxic and basic microenvironment. Moreover, these conditions are able to preserve soft tissues and favour mineralization. In consequence, the preservation model has to be rethinked at least for those cases where these microbial ecosystems were present.

10. Phototrophic microbial mats have shown to have a real effect in the early stages of fossilization in at least the two widely accepted processes for exceptional preservation: organic preservation and authigenic mineralization. In consequence, in the presence of mats the impact of these communities in fossilization should be capital.

11. The remarkable results herein exposed have opened new research lines in order to understand fossilization. The field of taphonomy is in need of more experiments to unveil, for instance, the mechanisms for preservation of the impressions or organic replicates made by microorganisms, or to understand how the minerals phases are determined and formed in tissues and how the environmental conditions would have an effect in this precipitation. Moreover, the knowledge of the microbial succession during the formation of the sarcophagus would be useful to understand the variation in the microenvironmental conditions.